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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 June 2001 (28.06.2001)

PCT

(10) International Publication Number
WO 01/45748 A1

- (51) International Patent Classification⁷: A61K 48/00, NJ 07065-0907 (US). FU, Tong-Ming [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
C12Q 1/70
- (21) International Application Number: PCT/US00/34724 (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (22) International Filing Date: 21 December 2000 (21.12.2000) (81) Designated States (*national*): AE, AG, AI., AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GI, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/171,542 22 December 1999 (22.12.1999) US (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
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- Published:
— With international search report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: POLYNUCLEOTIDE VACCINES EXPRESSING CODON OPTIMIZED HIV-1 POL AND MODIFIED HIV-1 POL

(57) Abstract: Pharmaceutical compositions which comprise HIV Pol DNA vaccines are disclosed, along with the production and use of these DNA vaccines. The pol-based DNA vaccines of the invention are administered directly introduced into living vertebrate tissue, preferably humans, and preferably express inactivated versions of the HIV Pol protein devoid of protease, reverse transcriptase activity, RNase H activity and integrase activity, inducing a cellular immune response which specifically recognizes human immunodeficiency virus-1 (HIV-1). The DNA molecules which comprise the open reading frame of these DNA vaccines are synthetic DNA molecules encoding codon optimized HIV-1 Pol and codon optimized inactive derivatives of optimized HIV-1 Pol, including DNA molecules which encode inactive Pol proteins which comprise an amino terminal leader peptide.

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TITLE OF THE INVENTION

POLYNUCLEOTIDE VACCINES EXPRESSING CODON OPTIMIZED HIV-1

5 POL AND MODIFIED HIV-1 POL

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S.
provisional application 60/171,542, filed December 22, 1999.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

15 REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates to HIV Pol polynucleotide pharmaceutical
20 products, as well as the production and use thereof which, when directly introduced
into living vertebrate tissue, preferably a mammalian host such as a human or a
non-human mammal of commercial or domestic veterinary importance, express the
HIV Pol protein or biologically relevant portions thereof within the animal, inducing a
cellular immune response which specifically recognizes human immunodeficiency
25 virus-1 (HIV-1). The polynucleotides of the present invention are synthetic DNA
molecules encoding codon optimized HIV-1 Pol and derivatives of optimized HIV-1
Pol, including constructs wherein protease, reverse transcriptase, RNase H and
integrase activity of HIV-1 Pol is inactivated. The polynucleotide vaccines of the
present invention should offer a prophylactic advantage to previously uninfected
30 individuals and/or provide a therapeutic effect by reducing viral load levels within an
infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element

(RRE). The Rev protein is promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper
5 T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its
10 single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load
15 within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or
20 latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark
25 of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the
30 disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine

development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

5 Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1
10 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a
15 prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets this need by disclosing a class of DNA vaccines based on host delivery and expression of modified versions of the HIV-1 gene, *pol*.

20 SUMMARY OF THE INVENTION

The present invention relates to synthetic DNA molecules (also referred to herein as "polynucleotides") and associated DNA vaccines (also referred to herein as "polynucleotide vaccines") which elicit cellular immune and humoral responses upon administration to the host, including primates and especially humans, and also
25 including a non-human mammal of commercial or domestic veterinary importance. An effect of the cellular immune-directed vaccines of the present invention should be the lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to
30 DNA vaccines which encode various forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized versions of wild type HIV-1 Pol, codon optimized versions of HIV-1 Pol fusion proteins, and codon optimized versions of HIV-1 Pol

proteins and fusion protein, including but not limited to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell.

5 A particular embodiment of the present invention relates to codon optimized wt-pol DNA constructs wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. The nucleotide sequence of a DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1 and the corresponding amino acid sequence of the expressed protein is
10 disclosed herein as SEQ ID NO:2.

The present invention preferably relates to a HIV-1 DNA pol construct which is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant may include but is not limited to a
15 mutated DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct
20 contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at
25 least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 2A-C) which has no PR, RT, RNase or IN activity, wherein three such point
30 mutations reside within each of the RT, RNase and IN catalytic domains. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the

preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as the leader peptide from human tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

The present invention especially relates to a HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide, such as the human tPA leader, at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, including but by no means limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 3. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8.

The present invention also relates to a substantially purified protein expressed from the DNA polynucleotide vaccines of the present invention, especially the purified

proteins set forth below as SEQ ID NOs: 2, 4, 6, and 8. These purified proteins may be useful as protein-based HIV vaccines.

The present invention also relates to non-codon optimized versions of DNA molecules and associated polynucleotides and associated DNA vaccines which
5 encode the various wild type and modified forms of the HIV Pol protein disclosed herein. Partial or fully codon optimized DNA vaccine expression vector constructs are preferred, but it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Pol which are shown to promote a substantial cellular immune and humoral
10 immune responses subsequent to host administration.

The DNA backbone of the DNA vaccines of the present invention are preferably DNA plasmid expression vectors. DNA plasmid expression vectors utilized in the present invention include but are not limited to constructs which comprise the cytomegalovirus promoter with the intron A sequence (CMV-intA) and
15 a bovine growth hormone transcription termination sequence. In addition, DNA plasmid vectors of the present invention preferably comprise an antibiotic resistance marker, including but not limited to an ampicillin resistance gene, a neomycin resistance gene or any other pharmaceutically acceptable antibiotic resistance marker. In addition, an appropriate polylinker cloning site and a prokaryotic origin of
20 replication sequence are also preferred. Specific DNA vectors exemplified herein include V1, V1J (SEQ ID NO:13), V1Jneo (SEQ ID NO:14), V1Jns (Figure 1A, SEQ ID NO:15), V1R (SEQ ID NO:26), and any of the aforementioned vectors wherein a nucleotide sequence encoding a leader peptide, preferably the human tPA leader, is fused directly downstream of the CMV-intA promoter, including but not limited to
25 V1Jns-tpa, as shown in Figure 1B and SEQ ID NO:28.

The present invention especially relates to a DNA vaccine and a pharmaceutically active vaccine composition which contains this DNA vaccine, and the use as prophylactic and/or therapeutic vaccine for host immunization, preferably human host immunization, against an HIV infection or to combat an existing HIV
30 condition. These DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and the essence of the present invention, biologically inactive Pol proteins (IA Pol; e.g., SEQ ID NO:4) devoid of significant PR, RT, RNase or IN

activity associated with wild type Pol and a concomitant construct which contains a leader peptide at the amino terminal region of the IA Pol protein. These constructs are ligated within an appropriate DNA plasmid vector, with or without a nucleotide sequence encoding a functional leader peptide. Preferred DNA vaccines of the present invention comprise codon optimized DNA molecules encoding codon optimized HIV-1 Pol and inactivated version of Pol, ligated in DNA vectors disclosed herein, or any of the aforementioned vectors wherein a nucleotide sequence encoding a leader peptide, preferably the human tPA leader, is fused directly downstream of the CMV-intA promoter, including but not limited to V1Jns-tpa, as shown in Figure 1B and SEQ ID NO:28.

Therefore, the present invention relates to DNA vaccines which include, but are in no way limited to V1Jns-WTPol (comprising the DNA molecule encoding WT Pol, as set forth in SEQ ID NO:2), V1Jns-tPA-WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), V1Jns-IAPol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and V1Jns-tPA-IAPol, (comprising the DNA molecule encoding tPA-IA Pol, as set forth in SEQ ID NO:8). Especially preferred are V1Jns-IAPol and V1Jns-tPA-IAPol, as exemplified in Example Section 2.

The present invention also relates to HIV Pol polynucleotide pharmaceutical products, as well as the production and use thereof, wherein the DNA vaccines are formulated with an adjuvant or adjuvants which may increase immunogenicity of the DNA polynucleotide vaccines of the present invention, namely by promoting an enhanced cellular and/or humoral response subsequent to inoculation. A preferred adjuvant is an aluminum phosphate-based adjuvant or a calcium phosphate based adjuvant, with an aluminum phosphate adjuvant being especially preferred. Another preferred adjuvant is a non-ionic block copolymer, preferably comprising the blocks of polyoxyethylene (POE) and polyoxypropylene (POP) such as a POE-POP-POE block copolymer. These adjuvanted forms comprising the DNA vaccines disclosed herein are useful in increasing cellular responses to DNA vaccination.

As used herein, a DNA vaccine or DNA polynucleotide vaccine is a DNA molecule (i.e., "nucleic acid", "polynucleotide") which contains essential regulatory elements such that upon introduction into a living, vertebrate cell, it is able to direct the cellular machinery to produce translation products encoded by the respective pol

genes of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A-B shows schematic representation of DNA vaccine expression
5 vectors V1Jns (A) and V1Jns-tPA (B) utilized for HIV-1 pol and HIV-1 modified pol constructs.

Figure 2A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as
10 listed in Table 1.

Figure 3 shows the codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-IA-Pol (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figure 4 shows generation of a humoral response (measured as the geometric means of anti-RT endpoint titers) from mice immunized with one or two doses of
15 codon optimized V1Jns-IApol and V1Jns-tpa-IApol. A portion of mice that received 30 ug of each plasmid was boosted at T=8 wks; sera from all mice were collected at 4 wk post dose 2.

Figure 5 shows the number of IFN-gamma secreting cells per 10e6 cells following stimulation with pools of either CD4⁺ (aa641-660, aa731-750) or CD8⁺
20 (aa201-220, aa311-330, aa571-590, aa781-800) specific peptides of splenocytes (pool of 5 spleens/cohort) from control mice and those vaccinated with increasing single dose of codon optimized V1Jns-IApol or 30 ug of codon optimized V1Jns-tpa-IApol (13 wks post dose 1). Mice (n=5) vaccinated with a second dose of 30 ug of either plasmid were analyzed in an Elispot assay at 6 wks post dose 2. Reported are the
25 sums of the number of spots stimulated by each individual CD8⁺ peptides because the spots in the wells to which the pool was added are too dense to acquire accurate counts. The CD4⁺ cell counts are taken from the responses to the peptide pool. Error bars represent standard deviations for counts from triplicate wells per sample per antigen.

Figure 6A-C shows ELispot analysis of peripheral blood cells collected from
30 rhesus macaques immunized three times (T=0, 4, 8 wks) with 5 mgs of codon optimized HIV-1 Pol expressing plasmids. Antigen-specific IFN-gamma secretion was stimulated by adding one of two pools consisting of 20-mer peptides derived from vaccine sequence (mpol-1, aa1-420; mpol-2, aa411-850). (A) Frequencies of

spot-forming cells (SFC) as a function of time for 3 monkeys (Tag No. 94R008, 94R013, 94R033) vaccinated with V1Jns-IApol. The reported values are corrected for background responses without peptide restimulation. (B) Frequencies of spot-forming cells (SFC) as a function of time for 3 monkeys (Tag No. 920078, 920073, 94R028) vaccinated with 5mgs of V1Jns-tpa-IApol. (C) ELISpot responses were also measured from a monkey (920072) that did not receive any immunization.

Figure 7A-B show bulk CTL killing from rhesus macaques immunized with codon optimized V1Jns-IApol (A) or codon optimized V1Jns-tpa-IApol (B) at 8 weeks following the third vaccination. Restimulation was performed using recombinant vaccinia virus expressing pol and target cells were prepared by pulsing with the peptide pools, mpol-1 and mpol-2.

Figure 8 shows detection of *in vitro* pol expression from cell lysates of 293 cells transfected with 10 ug of various pol constructs. Bands were detected using anti-serum from an HIV-1 seropositive human subject. Equal amounts of total protein were loaded for each lane. The lanes contain the lysates from cells transfected with the following: 1: mock; 2: V1Jns-wt-pol; 3: V1Jns-IApol (codon optimized); 4: V1Jns-tpa-IApol (codon optimized); 5: V1Jns-tpa-pol (codon optimized); 6: V1R-wt-pol (codon optimized); 7: blank; and 8: 80 ng RT.

Figure 9 shows the geometric mean anti-RT titers (GMT) plus the standard errors of the geometric means for cohorts of 5 mice that received one (open circles) or two doses (solid circles) of 1, 10, 100 μ g of V1R-wt-pol (codon optimized) or V1Jns-wt-pol. Sera from all animals were collected at 2 weeks post dose 2 (or 7 wks post dose 1) and assayed simultaneously. Statistical analyses were performed to compare cohorts that received the same amount and number of immunization of either plasmids; p values (two-tail) less than 5% are above the bars that connect the correlated cohorts to reflect statistically significant differences.

Figure 10 shows cellular immune responses in BALB/c mice vaccinated i.m. with 1 (pd1) or 2 (pd2) doses of varying amounts of either wt-pol (virus derived) or wt-pol (codon optimized) plasmids. At 3 wks post dose 2, frequencies of IFN- γ -secreting splenocytes are determined from pools of 5 spleens per cohort against mixtures of either CD4⁺ peptides (aa21-40, aa411-430, aa531-550, aa641-660, aa731-750, aa771-790) or CD8⁺ peptides (aa201-220, aa311-330) at 4 μ g/mL final concentration per peptide.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to synthetic DNA molecules and associated DNA vaccines which elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. An effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to DNA vaccines which encode various forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* constructs disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as a DNA vaccine. The HIV-1 genome employs predominantly uncommon codons compared to highly expressed human genes. Therefore, the *pol* open reading frame has been synthetically manipulated using optimal codons for human expression. As noted above, a preferred embodiment of the present invention relates to DNA molecules which comprise a HIV-1 *pol* open reading frame, whether encoding full length *pol* or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human.

The synthetic *pol* gene disclosed herein comprises the coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based on that of Hxb2r, a clonal isolate of IIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV *pol* DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-type (wt-*pol*) and inactivated *pol* (IA-*pol*) incorporates the use of human preferred ("humanized") codons for each amino acid

residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated DNA vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of the present invention relates to codon optimized wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

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AGATCTACCA TGGCCCCCAT CTCCCCCAT T GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
25 ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC
30 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
TCCCTTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGCCTGACC

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ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
 CCCGACAAGT GGAAGTGTGA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGAAGTGGT GATCCCCCTG
 5 ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
 CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
 10 GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
 GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGAAGTACAC CACCAACCAG
 AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
 15 GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
 GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
 ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
 CACTCCAACCT GGAGGGCTAT GGCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
 20 ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
 TGCTCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 25 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGG GTCCATGAAC
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTCAT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 CAGATCACCA AGATCCAGAA CTTGAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
 30 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACCTC
 GACATCAAGG TGGTGCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
 ID NO:1) .

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 5 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp
 10 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly
 15 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
 20 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
 25 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
 30 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala
 Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro

Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
 5 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
 10 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 15 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 20 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:2) .

The present invention especially relates to a codon optimized HIV-1 DNA pol
 construct wherein, in addition to deletion of the portion of the wild type sequence
 25 encoding the protease activity, a combination of active site residue mutations are
 introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed
 protein. Therefore, the present invention preferably relates to a HIV-1 DNA pol
 construct which is devoid of DNA sequences encoding any PR activity, as well as
 containing a mutation(s) which at least partially, and preferably substantially,
 30 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant may include
 but is not limited to a mutated DNA molecule comprising at least one nucleotide
 substitution which results in a point mutation which effectively alters an active site
 within the RT, RNase and/or IN regions of the expressed protein, resulting in at least
 substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of

HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 2A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

	<u>wt aa</u>	<u>aa residue</u>	<u>mutant aa</u>	<u>enzyme function</u>
25	Asp	112	Ala	RT
	Asp	187	Ala	RT
	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
30	Asp	626	Ala	IN
	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IAPol mutant vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IAPol":

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AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCACAAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTAAT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTTGTG
GGGGCTGAGA CTTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC

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ATCAGGAAGG TGCTGTTCCCT GGATGGCATT GACAAGGCC AGGATGAGCA TGAGAAGTAC
 CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
 ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
 TGCTCCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 5 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 10 GTGCAGATGG CTGTGTTTCAT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCTGTGG
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
 GACATCAAGG TGGTGCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 15 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
 NO:3).

In order to produce the IA-pol DNA vaccine construction, inactivation of the enzymatic functions was achieved by replacing a total of nine active-site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues

20 that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.* 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each

25 residue being substituted for an Ala residue, respectively (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-2119). Amino

30 acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4, as follows:
 Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys

Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg
Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
5 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
10 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly
Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
15 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
20 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
25 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala
Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala
Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
30 Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys

Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 5 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
 10 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 15 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations
 20 disclosed above may be suitable and therefore be utilized as an IA-pol-based vaccine
 of the present invention. For example, it may be possible to mutate only 2 of the 3
 residues within the respective reverse transcriptase, RNase H, and integrase coding
 regions while still abolishing these enzymatic activities. However, the IA-pol
 construct described above and disclosed as SEQ ID NO:3, as well as the expressed
 25 protein (SEQ ID NO:4) is preferred. It is also preferred that at least one mutation be
 present in each of the three catalytic domains.

Another aspect of the present invention is to generate codon optimized HIV-1
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal
 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide
 30 such as is found in highly expressed mammalian proteins such as immunoglobulin
 leader peptides. Any functional leader peptide may be tested for efficacy. However,
 a preferred embodiment of the present invention is to provide for HIV-1 Pol mutant
 vaccine constructions as disclosed herein which also comprise a leader peptide,
 preferably a leader peptide from human tPA. In other words, a codon optimized

HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As shown in Figure 1A-B for the DNA vector VIJns, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:28). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

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GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCGTGTGAA
GCTGAAGCCT GGCATGGATG GCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
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CCCCAGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
 GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
 GGGGGATGCC TACTTCTCTG TGCCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
 5 CATCCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
 GGGCTGGAAG GGCTCCCCCTG CCATCTTCCA GTCCCTCCATG ACCAAGATCC TGGAGCCCTT
 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
 TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG
 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
 10 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
 15 GCAGGGCCAG GGCCAGTGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT
 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CTGGTGAAG CTGTGGTACC AGCTGGAGAA
 20 GGAGCCCATT GTGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 GGAGGTGAAC ATTGTGACTG ACTCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 25 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA
 30 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGACAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA

CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
 GAACCCCTG TGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGTAT
 5 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

10 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 15 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 20 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 25 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 30 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln

Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 5 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 10 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 15 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu
 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn
 20 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 25 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 30 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant such
 as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal
 portion of the protein, which may effect cellular trafficking and hence,
 immunogenicity of the expressed protein within the host cell. Any such HIV-1 DNA

pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 3. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

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GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT
GGGGGATGCC TACTTCTCTG TGCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCCTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA

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GGGCTGGAAG GGCTCCCCCTG CCATCTTCCA GTCTTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG
GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
5 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC
CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
10 GCAGGGCCAG GGCCAGTGGG CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
TGGCAAGTAT GCCAGGATGA GGGGGGCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC
TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT
GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGACTGAG TACTGGCAGG CCACCTGGAT
CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
15 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA
GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
20 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
GGTGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGG AGGCCATGCA
TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA
25 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGCTACATT GAGGCTGAGG TGATCCCTGC
TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
GACCATCCAC ACTGCCAATG GCTCCAATT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
30 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGG CTGTGGTGTAT
CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA

TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID
NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as

5 follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
10 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser
15 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
20 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
25 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
30 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala

Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr
 5 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 10 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu
 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 15 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe
 20 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 25 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

The present invention also relates to a substantially purified protein expressed
 from the DNA polynucleotide vaccines of the present invention, especially the
 purified proteins set forth below as SEQ ID NOs: 2, 4, 6, and 8. These purified
 30 proteins may be useful as protein-based HIV vaccines.

The DNA backbone of the DNA vaccines of the present invention are
 preferably DNA plasmid expression vectors. DNA plasmid expression vectors are
 well known in the art and the present DNA vector vaccines may be comprised of any
 such expression backbone which contains at least a promoter for RNA polymerase

transcription, and a transcriptional terminator 3' to the HIV pol coding sequence. In one preferred embodiment, the promoter is the Rous sarcoma virus (RSV) long terminal repeat (LTR) which is a strong transcriptional promoter. A more preferred promoter is the cytomegalovirus promoter with the intron A sequence (CMV-intA).
5 A preferred transcriptional terminator is the bovine growth hormone terminator. In addition, to assist in large scale preparation of an HIV pol DNA vector vaccine, an antibiotic resistance marker is also preferably included in the expression vector. Ampicillin resistance genes, neomycin resistance genes or any other pharmaceutically acceptable antibiotic resistance marker may be used. In a preferred embodiment of
10 this invention, the antibiotic resistance gene encodes a gene product for neomycin resistance. Further, to aid in the high level production of the pharmaceutical by fermentation in prokaryotic organisms, it is advantageous for the vector to contain an origin of replication and be of high copy number. Any of a number of commercially available prokaryotic cloning vectors provide these benefits. In a preferred
15 embodiment of this invention, these functionalities are provided by the commercially available vectors known as pUC. It is desirable to remove non-essential DNA sequences. Thus, the lacZ and lacI coding sequences of pUC are removed in one embodiment of the invention.

DNA expression vectors which exemplify but in no way limit the present
20 invention are disclosed in PCT International Application No. PCT/US94/02751, International Publication No. WO 94/21797, hereby incorporated by reference. A first DNA expression vector is the expression vector pnRSV, wherein the rous sarcoma virus (RSV) long terminal repeat (LTR) is used as the promoter. A second embodiment relates to plasmid V1, a mutated pBR322 vector into which the CMV
25 promoter and the BGH transcriptional terminator is cloned. Another embodiment regarding DNA vector backbones relates to plasmid V1J. Plasmid V1J is derived from plasmid V1 and removes promoter and transcription termination elements in order to place them within a more defined context, create a more compact vector, and to improve plasmid purification yields. Therefore, V1J also contains the CMVintA
30 promoter and (BGH) transcription termination elements which control the expression of the HIV pol-based genes disclosed herein. The backbone of V1J is provided by pUC18. It is known to produce high yields of plasmid, is well-characterized by sequence and function, and is of minimum size. The entire *lac* operon was removed and the remaining plasmid was purified from an agarose electrophoresis gel,

blunt-ended with the T4 DNA polymerase, treated with calf intestinal alkaline phosphatase, and ligated to the CMVintA/BGH element. In a preferred DNA expression vector, the ampicillin resistance gene is removed from V1J and replaced with a neomycin resistance gene, to generate V1Jneo. An especially preferred DNA expression vector is V1Jns, which is the same as V1J except that a unique Sfi1 restriction site has been engineered into the single Kpn1 site at position 2114 of V1Jneo. The incidence of Sfi1 sites in human genomic DNA is very low (approximately 1 site per 100,000 bases). Thus, this vector allows careful monitoring for expression vector integration into host DNA, simply by Sfi1 digestion of extracted genomic DNA. Yet another preferred DNA expression vector used as the backbone to the HIV-1 pol-based DNA vaccines of the present invention is V1R. In this vector, as much non-essential DNA as possible is "trimmed" from the vector to produce a highly compact vector. This vector is a derivative of V1Jns. This vector allows larger inserts to be used, with less concern that undesirable sequences are encoded and optimizes uptake by cells when the construct encoding specific influenza virus genes is introduced into surrounding tissue. The specific DNA vectors of the present invention include but are not limited to V1, V1J (SEQ ID NO:13), V1Jneo (SEQ ID NO:14), V1Jns (Figure 1A, SEQ ID NO:15), V1R (SEQ ID NO:26), and any of the aforementioned vectors wherein a nucleotide sequence encoding a leader peptide, preferably the human tPA leader, is fused directly downstream of the CMV-intA promoter, including but not limited to V1Jns-tpa, as shown in Figure 1B and SEQ ID NO:28.

The present invention especially relates to a DNA vaccine and a pharmaceutically active vaccine composition which contains this DNA vaccine, and the use as prophylactic and/or therapeutic vaccine for host immunization, preferably human host immunization, against an HIV infection or to combat an existing HIV condition. These DNA vaccines are represented by codon optimized DNA molecules encoding HIV-1 Pol or biologically active Pol modifications or Pol-containing fusion proteins which are ligated within an appropriate DNA plasmid vector, with or without a nucleotide sequence encoding a functional leader peptide. DNA vaccines of the present invention may comprise codon optimized DNA molecules encoding HIV-1 Pol or biologically active Pol modifications or Pol-containing fusion proteins ligated in DNA vectors V1, V1J (SEQ ID NO:14), V1Jneo (SEQ ID NO:15), V1Jns (Figure 1A, SEQ ID NO:16), V1R (SEQ ID NO:26), or any of the aforementioned vectors

wherein a nucleotide sequence encoding a leader peptide, preferably the human tPA leader, is fused directly downstream of the CMV-intA promoter, including but not limited to V1Jns-tpa, as shown in Figure 1B and SEQ ID NO:28. To this end, polynucleotide vaccine constructions include , V1Jns-wtpol and V1R-wtpol (comprising the DNA molecule encoding WT Pol, as set forth in SEQ ID NO:2), V1Jns-tPA-WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), V1Jns-IAPol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and V1Jns-tPA-IAPol, (comprising the DNA molecule encoding tPA-IA Pol, as set forth in SEQ ID NO:8). Polynucleotide vaccine constructions V1R-wtpol, V1Jns-IAPol, and V1Jns-tPA-IAPol, are exemplified in Example Sections 3-5.

It will be evident upon review of the teaching within this specification that numerous vector/Pol antigen constructs may be generated. While the exemplified constructs are preferred, any number of vector/Pol antigen combinations are within the scope of the present invention, especially wild type or modified/inactivated Pol proteins which comprise at least one, preferably 5 or more and especially all nine mutations as shown in Table 1, with or without the inclusion of a leader sequence such as human tPA.

The DNA vector vaccines of the present invention may be formulated in any pharmaceutically effective formulation for host administration. Any such formulation may be, for example, a saline solution such as phosphate buffered saline (PBS). It will be useful to utilize pharmaceutically acceptable formulations which also provide long-term stability of the DNA vector vaccines of the present invention. During storage as a pharmaceutical entity, DNA plasmid vaccines undergo a physiochemical change in which the supercoiled plasmid converts to the open circular and linear form. A variety of storage conditions (low pH, high temperature, low ionic strength) can accelerate this process. Therefore, the removal and/or chelation of trace metal ions (with succinic or malic acid, or with chelators containing multiple phosphate ligands) from the DNA plasmid solution, from the formulation buffers or from the vials and closures, stabilizes the DNA plasmid from this degradation pathway during storage. In addition, inclusion of non-reducing free radical scavengers, such as ethanol or glycerol, are useful to prevent damage of the DNA plasmid from free radical production that may still occur, even in apparently demetalated solutions. Furthermore, the buffer type, pH, salt concentration, light

exposure, as well as the type of sterilization process used to prepare the vials, may be controlled in the formulation to optimize the stability of the DNA vaccine. Therefore, formulations that will provide the highest stability of the DNA vaccine will be one that includes a demetalated solution containing a buffer (phosphate or bicarbonate) with a pH in the range of 7-8, a salt (NaCl, KCl or LiCl) in the range of 100-200 mM, a metal ion chelator (e.g., EDTA, diethylenetriaminepenta-acetic acid (DTPA), malate, inositol hexaphosphate, tripolyphosphate or polyphosphoric acid), a non-reducing free radical scavenger (e.g. ethanol, glycerol, methionine or dimethyl sulfoxide) and the highest appropriate DNA concentration in a sterile glass vial, packaged to protect the highly purified, nuclease free DNA from light. A particularly preferred formulation which will enhance long term stability of the DNA vector vaccines of the present invention would comprise a Tris-HCl buffer at a pH from about 8.0 to about 9.0; ethanol or glycerol at about 3% w/v; EDTA or DTPA in a concentration range up to about 5 mM; and NaCl at a concentration from about 50 mM to about 500 mM. The use of such stabilized DNA vector vaccines and various alternatives to this preferred formulation range is described in detail in PCT International Application No. PCT/US97/06655 and PCT International Publication No. WO 97/40839, both of which are hereby incorporated by reference.

The DNA vector vaccines of the present invention may also be formulated with an adjuvant or adjuvants which may increase immunogenicity of the DNA polynucleotide vaccines of the present invention. A number of these adjuvants are known in the art and are available for use in a DNA vaccine, including but not limited to particle bombardment using DNA-coated gold beads, co-administration of DNA vaccines with plasmid DNA expressing cytokines, chemokines, or costimulatory molecules, formulation of DNA with cationic lipids or with experimental adjuvants such as saponin, monophosphoryl lipid A or other compounds which increase immunogenicity of the DNA vaccine. Another adjuvant for use in the DNA vector vaccines of the present invention are one or more forms of an aluminum phosphate-based adjuvant wherein the aluminum phosphate-based adjuvant possesses a molar PO_4/Al ratio of approximately 0.9. An additional mineral-based adjuvant may be generated from one or more forms of a calcium phosphate. These mineral-based adjuvants are useful in increasing cellular and humoral responses to DNA vaccination. These mineral-based compounds for use as DNA vaccines adjuvants are disclosed in PCT International

Application No. PCT/US98/02414, PCT International Publication No. WO 98/35562, which is hereby incorporated by reference. Another preferred adjuvant is a non-ionic block copolymer which shows adjuvant activity with DNA vaccines. The basic structure comprises blocks of polyoxyethylene (POE) and polyoxypropylene (POP) such as a POE-POP-POE block copolymer. Newman et al. (1998, *Critical Reviews in Therapeutic Drug Carrier Systems* 15(2): 89-142) review a class of non-ionic block copolymers which show adjuvant activity. The basic structure comprises blocks of polyoxyethylene (POE) and polyoxypropylene (POP) such as a POE-POP-POE block copolymer. Newman et al. *id.*, disclose that certain POE-POP-POE block copolymers may be useful as adjuvants to an influenza protein-based vaccine, namely higher molecular weight POE-POP-POE block copolymers containing a central POP block having a molecular weight of over about 9000 daltons to about 20,000 daltons and flanking POE blocks which comprise up to about 20% of the total molecular weight of the copolymer (see also U.S. Reissue Patent No. 36,665, U.S. Patent No. 5,567,859, U.S. Patent No. 5,691,387, U.S. Patent No. 5,696,298 and U.S. Patent No. 5,990,241, all issued to Emanuele, et al., regarding these POE-POP-POE block copolymers). WO 96/04932 further discloses higher molecular weight POE/POP block copolymers which have surfactant characteristics and show biological efficacy as vaccine adjuvants. The above cited references within this paragraph are hereby incorporated by reference in their entirety. It is therefore within the purview of the skilled artisan to utilize available adjuvants which may increase the immune response of the polynucleotide vaccines of the present invention in comparison to administration of a non-adjuvanted polynucleotide vaccine.

The DNA vector vaccines of the present invention are administered to the host by any means known in the art, such as enteral and parenteral routes. These routes of delivery include but are not limited to intramuscular injection, intraperitoneal injection, intravenous injection, inhalation or intranasal delivery, oral delivery, sublingual administration, subcutaneous administration, transdermal administration, transcutaneous administration, percutaneous administration or any form of particle bombardment, such as a biolistic device such as a "gene gun" or by any available needle-free injection device. The preferred methods of delivery of the HIV-1 Pol-based DNA vaccines disclosed herein are intramuscular injection, subcutaneous administration and needle-free injection. An especially preferred method is

intramuscular delivery.

The amount of expressible DNA to be introduced to a vaccine recipient will depend on the strength of the transcriptional and translational promoters used in the DNA construct, and on the immunogenicity of the expressed gene product. In
5 general, an immunologically or prophylactically effective dose of about 1 μ g to greater than about 20 mg, and preferably in doses from about 1 mg to about 5 mg is administered directly into muscle tissue. As noted above, subcutaneous injection, intradermal introduction, impression through the skin, and other modes of
10 administration such as intraperitoneal, intravenous, inhalation and oral delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided in a fashion which optimizes the overall immune response to the Pol-based DNA vector vaccines of the present invention.

The aforementioned polynucleotides, when directly introduced into a vertebrate *in vivo*, express the respective HIV-1 Pol protein within the animal and in
15 turn induce a cellular immune response within the host to the expressed Pol antigen. To this end, the present invention also relates to methods of using the HIV-1 Pol-based polynucleotide vaccines of the present invention to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the
20 acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As noted above, the present invention contemplates a method of administration or use of the DNA pol-based vaccines of the present invention using any of the known routes of introducing polynucleotides into living tissue to induce expression of proteins.

25 Therefore, the present invention provides for methods of using a DNA pol-based vaccine utilizing the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of these DNA pol-based vaccines. This intracellular expression of the Pol-based immunogen induces a cellular immune
30 response which provides a substantial level of protection against an existing HIV-1 infection or provides a substantial level of protection against a future infection in a presently uninfected host.

The following examples are provided to illustrate the present invention without, however, limiting the same hereto.

EXAMPLE 1

Vaccine Vectors

VI – Vaccine vector V1 was constructed from pCMVIE-AKI-DHFR (Whang et al., 1987, *J. Virol.* 61: 1796). The AKI and DHFR genes were removed by cutting the vector with EcoRI and self-ligating. This vector does not contain intron A in the CMV promoter, so it was added as a PCR fragment that had a deleted internal SacI site [at 1855 as numbered in Chapman, et al., 1991, *Nuc. Acids Res.* 19: 3979). The template used for the PCR reactions was pCMVintA-Lux, made by ligating the HindIII and NheI fragment from pCMV6a120 (see Chapman et al., *ibid.*), which includes hCMV-IE1 enhancer/promoter and intron A, into the HindIII and XbaI sites of pBL3 to generate pCMVIntBL. The 1881 base pair luciferase gene fragment (HindIII-SmaI Klenow filled-in) from RSV-Lux (de Wet et al., 1987, *Mol. Cell Biol.* 7: 725) was ligated into the SalI site of pCMVIntBL, which was Klenow filled-in and phosphatase treated. The primers that spanned intron A are: 5' primer: 5'-CTATAT AAGCAGAGCTCGTTTAG-3' (SEQ ID NO:10); 3' primer: 5'-GTAGCAAA GATCTAAGGACGGTGACTGCAG-3' (SEQ ID NO:11). The primers used to remove the SacI site are: sense primer, 5'-GTATGTGTCTGAAAATGAGCG TGGAGATTGGGCTCGCAC-3' (SEQ ID NO:12) and the antisense primer, 5'-GTGCGAGCCCAATCTCCACGCTCATTTTCAGAC ACATAC-3' (SEQ ID NO:13). The PCR fragment was cut with Sac I and Bgl II and inserted into the vector which had been cut with the same enzymes.

V1J – Vaccine vector V1J was generated to remove the promoter and transcription termination elements from vector V1 in order to place them within a more defined context, create a more compact vector, and to improve plasmid purification yields. V1J is derived from vectors V1 and pUC18, a commercially available plasmid. V1 was digested with SspI and EcoRI restriction enzymes producing two fragments of DNA. The smaller of these fragments, containing the CMVintA promoter and Bovine Growth Hormone (BGH) transcription termination elements which control the expression of heterologous genes, was purified from an agarose electrophoresis gel. The ends of this DNA fragment were then "blunted" using the T4 DNA polymerase enzyme in order to facilitate its ligation to another "blunt-ended" DNA fragment. pUC18 was chosen to provide the "backbone" of the expression vector. It is known to produce high yields of plasmid, is well-

characterized by sequence and function, and is of small size. The entire *lac* operon was removed from this vector by partial digestion with the HaeII restriction enzyme. The remaining plasmid was purified from an agarose electrophoresis gel, blunt-ended with the T4 DNA polymerase treated with calf intestinal alkaline phosphatase, and
 5 ligated to the CMVintA/BGH element described above. Plasmids exhibiting either of two possible orientations of the promoter elements within the pUC backbone were obtained. One of these plasmids gave much higher yields of DNA in *E. coli* and was designated V1J. This vector's structure was verified by sequence analysis of the
 10 junction regions and was subsequently demonstrated to give comparable or higher expression of heterologous genes compared with V1. The nucleotide sequence of V1J is as follows:

TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
 CAGCTTGCTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
 15 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG
 CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT AATCAATTAC
 GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG
 CCCGCCTGGC TGACCGCCCA ACGACCCCGG CCCATTGACG TCAATAATGA CGTATGTTCC
 20 CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC
 TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC
 TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
 CATCAATGGG CGTGGATAGC GGTGTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
 25 CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA
 TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA CGGTGCATTG GAACGCGGAT
 TCCCCGTGCC AAGAGTGACG TAAGTACCGC CTATAGAGTC TATAGGCCCA CCCCTTGGC
 30 TTCTTATGCA TGCTATACTG TTTTGGCTT GGGGTCTATA CACCCCGCT TCCTCATGTT
 ATAGGTGATG GTATAGCTTA GCCTATAGGT GTGGGTATT GACCATTATT GACCACTCCC
 CTATTGGTGA CGATACTTTC CATTACTAAT CCATAACATG GCTCTTTGCC ACAACTCTCT
 TTATTGGCTA TATGCCAATA CACTGTCCTT CAGAGACTGA CACGGACTCT GTATTTTAC
 AGGATGGGGT CTCATTTATT ATTTACAAAT TCACATATAC AACACCACCG TCCCCAGTGC

CCGCAGTTTT TATTAAACAT AACGTGGGAT CTCCACGCGA ATCTCGGGTA CGTGTTCCGG
ACATGGGCTC TTCTCCGGTA GCGGCGGAGC TTCTACATCC GAGCCCTGCT CCCATGCCTC
CAGCGACTCA TGGTCGCTCG GCAGCTCCTT GCTCCTAACA GTGGAGGCCA GACTTAGGCA
CAGCACGATG CCCACCACCA CCAGTGTGCC GCACAAGGCC GTGGCGGTAG GGTATGTGTC
5 TGAAAATGAG CTCGGGGAGC GGGCTTGCAC CGCTGACGCA TTTGGAAGAC TTAAGGCAGC
GGCAGAAGAA GATGCAGGCA GCTGAGTTGT TGTGTTCTGA TAAGAGTCAG AGGTAACTCC
CGTTGCGGTG CTGTTAACGG TGGAGGGCAG TGTAGTCTGA GCAGTACTCG TTGCTGCCGC
GCGCGCCACC AGACATAATA GCTGACAGAC TAACAGACTG TTCCTTTCCA TGGGTCTTTT
CTGCAGTCAC CGTCCTTAGA TCTGCTGTGC CTTCTAGTTG CCAGCCATCT GTTGTTTGCC
10 CCTCCCCGT GCCTTCCTTG ACCCTGGAAG GTGCCACTCC CACTGTCCTT TCCTAATAAA
ATGAGGAAAT TGCATCGCAT TGTCTGAGTA GGTGTCATTC TATTCTGGGG GGTGGGGTGG
GGCAGCACAG CAAGGGGGAG GATTGGGAAG ACAATAGCAG GCATGCTGGG GATGCGGTGG
GCTCTATGGG TACCCAGGTG CTGAAGAATT GACCCGGTTC CTCCTGGGCC AGAAAGAAGC
AGGCACATCC CCTTCTCTGT GACACACCCT GTCCACGCCC CTGGTTCCTTA GTTCCAGCCC
15 CACTCATAGG AACTCATAG CTCAGGAGGG CTCCGCCTTC AATCCCACCC GCTAAAGTAC
TTGGAGCGGT CTCTCCCTCC CTCATCAGCC CACCAAACCA AACCTAGCCT CCAAGAGTGG
GAAGAAATTA AAGCAAGATA GGCTATTAAAG TGCAAGAGGA GAGAAAATGC CTCCAACATG
TGAGGAAGTA ATGAGAGAAA TCATAGAATT TCTTCCGCTT CCTCGCTCAC TGA CTGCTG
CGCTCGGTGCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA
20 TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAGGCC
AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTCCATA GGCTCCGCC CCCTGACGAG
CATCACAAAA ATCAGCGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC
CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT GCCGCTTACC
GGATACCTGT CCGCCTTTCT CCCTTCGGA AGCGTGGCGC TTTCTCAATG CTCACGCTGT
25 AGGTATCTCA GTTCGGTGTA GGTGTTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC
GTTACGCCCC ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGTAAGA
CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAAACAGGA TTAGCAGAGC GAGGTATGTA
GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG AAGGACAGTA
TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA
30 TCCGGCAAAC AAACCACCGC TGGTAGCGGT GGTTTTTTTT TTTGCAAGCA GCAGATTACG
CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC TGACGCTCAG
TGGAACGAAA ACTCACGTTA AGGATTTTGT GTCATGAGAT TATCAAAAAG GATCTTCACC
TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA TGAGTAACT
TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT

CGTTCATCCA TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA
 CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA
 TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTTATCC
 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT
 5 AGTTTGC GCA ACGTTGTTGC CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT
 ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCAAGGCGAG TTACATGATC CCCCATGTTG
 TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA
 GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC TTACTGTCAT GCCATCCGTA
 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA GTGTATGCGG
 10 CGACCGAGTT GCTCTTGCCC GCGCTCAATA CGGGATAATA CCGCGCCACA TAGCAGAACT
 TTAAGAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG
 CTGTTGAGAT CCAGTTTCAT GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT
 ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA
 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTCAATA TTATTGAAGC
 15 ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA
 CAAATAGGGG TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT
 ATTATCATGA CATTAACCTA TAAAAATAGG CGTATCACGA GGCCCTTTCG TC (SEQ ID
 NO:14) .

V1Jneo – Construction of vaccine vector *V1Jneo* expression vector involved
 20 removal of the *amp^r* gene and insertion of the *kan^r* gene (neomycin
 phosphotransferase). The *amp^r* gene from the pUC backbone of *V1J* was removed by
 digestion with *SspI* and *Eam1105I* restriction enzymes. The remaining plasmid was
 purified by agarose gel electrophoresis, blunt-ended with T4 DNA polymerase, and
 then treated with calf intestinal alkaline phosphatase. The commercially available
 25 *kan^r* gene, derived from transposon 903 and contained within the pUC4K plasmid,
 was excised using the *PstI* restriction enzyme, purified by agarose gel electrophoresis,
 and blunt-ended with T4 DNA polymerase. This fragment was ligated with the *V1J*
 backbone and plasmids with the *kan^r* gene in either orientation were derived which
 were designated as *V1Jneo* #'s 1 and 3. Each of these plasmids was confirmed by
 30 restriction enzyme digestion analysis, DNA sequencing of the junction regions, and
 was shown to produce similar quantities of plasmid as *V1J*. Expression of
 heterologous gene products was also comparable to *V1J* for these *V1Jneo* vectors.
V1Jneo#3, referred to as *V1Jneo* hereafter, was selected which contains the *kan^r* gene
 in the same orientation as the *amp^r* gene in *V1J* as the expression construct and

provides resistance to neomycin, kanamycin and G418. The nucleotide sequence of V1Jneo is as follows:

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TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
5 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG
CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT AATCAATTAC
GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG
10 CCCGCCCTGGC TGACCGCCCA ACGACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC
CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC
TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC
TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
15 CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
CTCCGCCCCA TTGACGCAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA
TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA CGGTGCATTG GAACGCGGAT
20 TCCCCGTGCC AAGAGTGACG TAAGTACCGC CTATAGAGTC TATAGGCCCA CCCCTTGGC
TTCTTATGCA TGCTATACTG TTTTGGCTT GGGGTCTATA CACCCCGCT TCCTCATGTT
ATAGGTGATG GTATAGCTTA GCCTATAGGT GTGGGTATT GACCATTATT GACCACTCCC
CTATTGGTGA CGATACTTC CATTACTAAT CCATAACATG GCTCTTTGCC ACAACTCTCT
TTATTGGCTA TATGCCAATA CACTGTCCTT CAGAGACTGA CACGGACTCT GTATTTTAC
25 AGGATGGGGT CTCATTTATT ATTTACAAAT TCACATATAC AACACCACCG TCCCAGTGC
CCGCAGTTTT TATTAAACAT AACGTGGGAT CTCCACGCGA ATCTCGGGTA CGTGTTCGGG
ACATGGGCTC TTCTCCGGTA GCGGCGGAGC TTCTACATCC GAGCCCTGCT CCCATGCCTC
CAGCGACTCA TGGTCGCTCG GCAGCTCCTT GCTCCTAACA GTGGAGGCCA GACTTAGGCA
CAGCACGATG CCCACCACCA CCAGTGTGCC GCACAAGGCC GTGGCGGTAG GGTATGTGTC
30 TGAAAATGAG CTCGGGGAGC GGGCTTGAC CGCTGACGCA TTTGGAAGAC TTAAGGCAGC
GGCAGAAGAA GATGCAGGCA GCTGAGTTGT TGTGTTCTGA TAAGAGTCAG AGGTAACTCC
CGTTGCGGTG CTGTTAACGG TGGAGGGCAG TGTAAGTCTGA GCAGTACTCG TTGCTGCCGC
GCGCGCCACC AGACATAATA GCTGACAGAC TAACAGACTG TTCCTTTCCA TGGGTCTTTT
CTGCAGTCAC CGTCCTTAGA TCTGCTGTGC CTTCTAGTTG CCAGCCATCT GTTGTTTGCC

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CCTCCCCCGT GCCTTCCTTG ACCCTGGAAG GTGCCACTCC CACTGTCCTT TCCTAATAAA
ATGAGGAAAT TGCATCGCAT TGTCTGAGTA GGTGTCATTC TATTCTGGGG GGTGGGGTGG
GGCAGCACAG CAAGGGGGAG GATTGGGAAG ACAATAGCAG GCATGCTGGG GATGCCGTGG
GCTCTATGGG TACCCAGGTG CTGAAGAATT GACCCGGTTC CTCCTGGGCC AGAAAGAAGC
5 AGGCACATCC CCTTCTCTGT GACACACCCCT GTCCACGCC CTGGTTCTTA GTTCCAGCCC
CACTCATAGG AACTTCATAG CTCAGGAGGG CTCCGCCCTTC AATCCCACCC GCTAAAGTAC
TTGGAGCGGT CTCTCCCTCC CTCATCAGCC CACCAAACCA AACCTAGCCT CCAAGAGTGG
GAAGAAATTA AAGCAAGATA GGCTATTAAG TGCAGAGGGA GAGAAAATGC CTCCAACATG
TGAGGAAGTA ATGAGAGAAA TCATAGAATT TCTTCCGCTT CCTCGCTCAC TGACTCGCTG
10 CGCTCGGTG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA
TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAGGCC
AGGAACCGTA AAAAGGCCGC GTTGTGGCG TTTTCCATA GGCTCCGCCC CCCTGACGAG
CATCAAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC
CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT GCCGCTTACC
15 GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCAATG CTCACGCTGT
AGGTATCTCA GTTCGGTGTA GGTGTTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC
GTTACGCCC ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGGTAAGA
CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC GAGGTATGTA
GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG AAGGACAGTA
20 TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA
TCCGGCAAAC AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACC
CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC TGACGCTCAG
TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG GATCTTCACC
TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA TGAGTAAACT
25 TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT
CGTTCATCCA TAGTTGCCTG ACTCCGGGGG GGGGGGGCGC TGAGGTCTGC CTCGTGAAGA
AGGTGTTGCT GACTCATACC AGGCCTGAAT CGCCCCATCA TCCAGCCAGA AAGTGAGGGA
GCCACGGTTG ATGAGAGCTT TGTTGTAGGT GGACCAGTTG GTGATTTTGA ACTTTTGCTT
TGCCACGGAA CGGTCTGCGT TGTCGGGAAG ATGCGTGATC TGATCCTTCA ACTCAGCAAA
30 AGTTCGATTT ATTCAACAAA GCCGCCGTCC CGTCAAGTCA GCGTAATGCT CTGCCAGTGT
TACAACCAAT TAACCAATTC TGATTAGAAA AACTCATCGA GCATCAAAATG AAATGCAAT
TTATTCATAT CAGGATTATC AATACCATAT TTTTGAAAAA GCCGTTTCTG TAATGAAGGA
GAAAACTCAC CGAGGCAGTT CCATAGGATG GCAAGATCCT GGTATCGGTC TGCGATTCCG
ACTCGTCCAA CATCAATACA ACCTATTAAT TTCCCTCGT CAAAAATAAG GTTATCAAGT

GAGAAATCAC CATGAGTGAC GACTGAATCC GGTGAGAATG GCAAAAGCTT ATGCATTTCT
 TTCCAGACTT GTTCAACAGG CCAGCCATTA CGCTCGTCAT CAAAATCACT CGCATCAACC
 AAACCGTTAT TCATTCTGTA TTGCGCCTGA GCGAGACGAA ATACGCGATC GCTGTTAAAA
 GGACAATTAC AAACAGGAAT CGAATGCAAC CGGCGCAGGA ACACTGCCAG CGCATCAACA
 5 ATATTTTCAC CTGAATCAGG ATATTCTTCT AATACCTGGA ATGCTGTTTT CCCGGGGATC
 GCAGTGGTGA GTAACCATGC ATCATCAGGA GTACGGATAA AATGCTTGAT GGTGGAAGA
 GGCATAAATT CCGTCAGCCA GTTAGTCTG ACCATCTCAT CTGTAACATC ATTGGCAACG
 CTACCTTTGC CATGTTTCAG AAACAACCTCT GGCGCATCGG GCTTCCCATA CAATCGATAG
 ATTGTCGCAC CTGATTGCCC GACATTATCG CGAGCCCATT TATACCCATA TAAATCAGCA
 10 TCCATGTTGG AATTTAATCG CGGCCTCGAG CAAGACGTTT CCCGTTGAAT ATGGCTCATA
 ACACCCCTTG TATTACTGTT TATGTAAGCA GACAGTTTTA TTGTTTCATGA TGATATATTT
 TTATCTTGTC CAATGTAACA TCAGAGATTT TGAGACACAA CGTGGCTTTC CCCCCCCCCC
 CATTATTGAA GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT
 TAGAAAAATA AACAAATAGG GGTTCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC
 15 TAAGAAACCA TTATTATCAT GACATTAACC TATAAAAATA GGCGTATCAC GAGGCCCTTT
 CGTC (SEQ ID NO:15).

V1Jns - The expression vector *V1Jns* was generated by adding an *SfiI* site to *V1Jneo* to facilitate integration studies. A commercially available 13 base pair *SfiI* linker (New England BioLabs) was added at the *KpnI* site within the BGH sequence
 20 of the vector. *V1Jneo* was linearized with *KpnI*, gel purified, blunted by T4 DNA polymerase, and ligated to the blunt *SfiI* linker. Clonal isolates were chosen by restriction mapping and verified by sequencing through the linker. The new vector was designated *V1Jns*. Expression of heterologous genes in *V1Jns* (with *SfiI*) was comparable to expression of the same genes in *V1Jneo* (with *KpnI*).

25 The nucleotide sequence of *V1Jns* is as follows:

TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGTCCCG GAGACGGTCA
 CAGCTTGCTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG
 30 CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT AATCAATTAC
 GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG
 CCCGCCTGGC TGACCGCCCA ACGACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC
 CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC

TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC
 TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
 CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
 5 CGTCAATGGG AGTTTGT TTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA
 TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA CGGTGCATTG GAACGCGGAT
 TCCCCGTGCC AAGAGTGACG TAAGTACCGC CTATAGACTC TATAGGCACA CCCCTTTGGC
 10 TCTTATGCAT GCTATACTGT TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA
 TAGGTGATGG TATAGCTTAG CCTATAGGTG TGGGTATTG ACCATTATTG ACCACTCCCC
 TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA CAACTATCTC
 TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC ACGGACTCTG TATTTTACAA
 GGATGGGGTC CCATTATTAT TTTACAAATT CACATATACA ACAACGCCGT CCCCCGTGCC
 15 CGCAGTTTTT ATTAAACATA GCGTGGGATC TCCACGGAA TCTCGGGTAC GTGTTCCGGA
 CATGGGCTCT TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
 AGCGGCTCAT GGTGCTCGG CAGCTCCTTG CTCTAACAG TGGAGGCCAG ACTTAGGCAC
 AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG TGGCGGTAGG GTATGTGTCT
 GAAAAATGAGC GTGGAGATTG GGCTCGCACG GCTGACGCAG ATGGAAGACT TAAGGCAGCG
 20 GCAGAAGAAG ATGCAGGCAG CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACCTCC
 GTTGGCGTGC TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCCGG
 CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT GGGTCTTTTC
 TGCAGTCACC GTCCTTAGAT CTGCTGTGCC TTCTAGTTGC CAGCCATCTG TTGTTTGCCC
 CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCTTTT CCTAATAAAA
 25 TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTATTCT ATTCTGGGGG GTGGGGTGGG
 GCAGGACAGC AAGGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG ATGCGGTGGG
 CTCTATGGCC GCTGCGGCCA GGTGCTGAAG AATTGACCCG GTTCCTCCTG GGCCAGAAAG
 AAGCAGGCAC ATCCCCTTCT CTGTGACACA CCCTGTCCAC GCCCCTGGTT CTTAGTTCCA
 GCCCCACTCA TAGGACACTC ATAGCTCAGG AGGGCTCCGC CTTCAATCCC ACCCGCTAAA
 30 GTACTTGAG CGGTCTCTCC TCCTCTCATC AGCCACCAA ACCAAACCTA GCCTCCAAGA
 GTGGGAAGAA ATTAAAGCAA GATAGGCTAT TAAGTGCAGA GGGAGAGAAA ATGCCTCCAA
 CATGTGAGGA AGTAATGAGA GAAATCATAG AATTTCTTCC GCTTCCTCGC TCACTGACTC
 GCTGCGCTCG GTCGTTCCGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG
 GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA

GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCCTTTTTTC CATAGGCTCC GCCCCCTGA
 CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG
 ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCCTCTT CCTGTTCCGA CCCTGCCGCT
 TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGAAGCGTG GCGCTTTCTC ATAGCTCAGC
 5 CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC
 CCCCCTTCAG CCCGACCGCT GCGCCTTATC CGGTAACAT CTCTTTGAGT CCAACCCGGT
 AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA
 TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC
 AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC
 10 TTGATCCGGC AAACAAACCA CCGCTGGTAG CCGTGGTTTT TTTGTTTGCA AGCAGCAGAT
 TACGCGCAGA AAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTCTACGG GGTCTGACGC
 TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT
 CACCTAGATC CTTTTAAAT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA
 AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG CGATCTGTCT
 15 ATTTCTGTTCA TCCATAGTTG CCTGACTCGG GGGGGGGGGG CGCTGAGGTC TGCTCTGTGA
 AGAAGGTGTT GCTGACTCAT ACCAGGCCTG AATCGCCCCA TCATCCAGCC AGAAAGTGAG
 GGAGCCACGG TTGATGAGAG CTTTGTGTGA GGTGGACCAG TTGGTGATTT TGAACCTTTG
 CTTTGCCACG GAACGGTCTG CGTTGTCTGG AAGATGCGTG ATCTGATCCT TCAACTCAGC
 AAAAGTTCGA TTTATTCAAC AAAGCCGCCG TCCCGTCAAG TCAGCGTAAT GCTCTGCCAG
 20 TGTTACAACC AATTAACCAA TTCTGATTAG AAAAATCAT CGAGCATCAA ATGAAACTGC
 AATTTATTCA TATCAGGATT ATCAATACCA TATTTTGTGA AAAGCCGTTT CTGTAATGAA
 GGAGAAACT CACCGAGGCA GTTCCATAGG ATGGCAAGAT CCTGGTATCG GTCTGCGATT
 CCGACTCGTC CAACATCAAT ACAACCTATT AATTTCCCCT CGTCAAAAAT AAGGTTATCA
 AGTGAGAAAT CACCATGAGT GACGACTGAA TCCGGTGAGA ATGGCAAAAG CTTATGCATT
 25 TCTTTCCAGA CTTGTTCAAC AGGCCAGCCA TTACGCTCGT CATCAAAATC ACTCGCATCA
 ACCAAACCGT TATTCATTCG TGATTGCGCC TGAGCGAGAC GAAATACGCG ATCGCTGTTA
 AAAGGACAAT TACAAACAGG AATCGAATGC AACCGGCGCA GGAACACTGC CAGCGCATCA
 ACAATATTTT CACCTGAATC AGGATATTCT TCTAATACCT GGAATGCTGT TTCCCCGGG
 ATCGCAGTGG TGAGTAACCA TGCATCATCA GGAGTACGGA TAAAATGCTT GATGGTCGGA
 30 AGAGGCATAA ATTCCGTCAG CCAGTTTAGT CTGACCATCT CATCTGTAAC ATCATTTGGA
 ACGCTACCTT TGCCATGTTT CAGAAACAAC TCTGGCGCAT CGGGCTTCCC ATACAATCGA
 TAGATTGTCG CACCTGATTG CCCGACATTA TCGCGAGCCC ATTTATACCC ATATAAATCA
 GCATCCATGT TGAATTTTAA TCGCGGCTC GAGCAAGACG TTTCCCGTTG AATATGGCTC
 ATAACACCCC TTGTATTACT GTTTATGTAA GCAGACAGTT TTATTGTTCA TGATGATATA

TTTTTATCTT GTGCAATGTA ACATCAGAGA TTTTGAGACA CAACGTGGCT TTCCCCCCCC
 CCCCATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAATGT
 ATTTAGAAAA ATAAACAAAT AGGGGTTCG CGCACATTTC CCCGAAAAGT GCCACCTGAC
 GTCTAAGAAA CCATTATTAT CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC
 5 TTTTCGTC (SEQ ID NO:16).

The underlined nucleotides of SEQ ID NO:16 represent the SfiI site introduced into the Kpn I site of V1Jneo.

V1Jns-tPA – The vaccine vector V1Jns-tPA was constructed in order to fuse an heterologous leader peptide sequence to the pol DNA constructs of the present invention. More specifically, the vaccine vector V1Jns was modified to include the human tissue-specific plasminogen activator (tPA) leader. As an exemplification, but by no means a limitation of generating a pol DNA construct comprising an amino-terminal leader sequence, plasmid V1Jneo was modified to include the human tissue-specific plasminogen activator (tPA) leader. Two synthetic complementary oligomers were annealed and then ligated into V1Jneo which had been BglII digested. The sense and antisense oligomers were 5'-GATCACCATGGATGCAATGAAGAG AGGGCTCTGCTGTGTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAG CGA-3' (SEQ ID NO:17); and, 5'-GATCTCGCTGGGCGAAACGAAGACTGCTCC ACACAGCAGCAGCACACAGCAGAGCCCTCTCTTCATTGCATCCATGGT-3' (SEQ ID NO:18). The Kozak sequence is underlined in the sense oligomer. These oligomers have overhanging bases compatible for ligation to BglII-cleaved sequences. After ligation the upstream BglII site is destroyed while the downstream BglII is retained for subsequent ligations. Both the junction sites as well as the entire tPA leader sequence were verified by DNA sequencing. Additionally, in order to conform with V1Jns (=V1Jneo with an SfiI site), an SfiI restriction site was placed at the KpnI site within the BGH terminator region of V1Jneo-tPA by blunting the KpnI site with T4 DNA polymerase followed by ligation with an SfiI linker (catalogue #1138, New England Biolabs), resulting in V1Jns-tPA. This modification was verified by restriction digestion and agarose gel electrophoresis.

30 The V1Jns-tpa vector nucleotide sequence is as follows:

TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGTCCCG GAGACGGTCA
 CAGCTTGCTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG

CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT AATCAATTAC
 GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG
 CCCGCCGTCG TGACCGCCCA ACGACCCCGC CCCATTGACG TCAATAATGA CGTATGTTCC
 5 CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC
 TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC
 TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
 CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
 10 CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA
 TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA CGGTGCATTG GAACGCGGAT
 TCCCCGTGCC AAGAGTGACG TAAGTACCGC CTATAGACTC TATAGGCACA CCCCTTTGGC
 15 TCTTATGCAT GCTATACTGT TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA
 TAGGTGATGG TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
 TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA CAACTATCTC
 TATTGGCTAT ATGCCAATAC TCTGTCTTC AGAGACTGAC ACGGACTCTG TATTTTTACA
 GGATGGGGTC CCATTTATTA TTTACAAATT CACATATACA ACAACGCCGT CCCCCGTGCC
 20 CGCAGTTTTT ATTAAACATA GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA
 CATGGGCTCT TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
 AGCGGCTCAT GGTGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG ACTTAGGCAC
 AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG TGGCGGTAGG GTATGTGTCT
 GAAAATGAGC GTGGAGATTG GGCTCGCACG GCTGACGCAG ATGGAAGACT TAAGGCAGCG
 25 GCAGAAGAAG ATGCAGGCAG CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACCTCC
 GTTGCGGTGC TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
 CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT GGGTCTTTTC
 TGCAGTCACC GTCCTTAGAT CACCATGGAT GCAATGAAGA GAGGGCTCTG CTGTGTGCTG
CTGTGTGTG GAGCAGTCTT CGTTTCGCCC AGCGAGATCT GCTGTGCCTT CTAGTTGCCA
 30 GCCATCTGTT GTTTGCCCCCT CCCCCGTGCC TTCCTTGACC CTGGAAGGTG CCACTCCCAC
 TGTCCTTTCC TAATAAAATG AGGAAATTGC ATCGCATTGT CTGAGTAGGT GTCATTCTAT
 TCTGGGGGGT GGGGTGGGGC AGGACAGCAA GGGGGAGGAT TGGGAAGACA ATAGCAGGCA
 TGCTGGGGAT GCGGTGGGCT CTATGGCCGC TGCGGCCAGG TGCTGAAGAA TTGACCCGGT
 TCCTCCTGGG CCAGAAAGAA GCAGGCACAT CCCCTTCTCT GTGACACACC CTGTCCACGC

CCCTGGTTCT TAGTTCAGC CCCACTCATA GGACACTCAT AGCTCAGGAG GGCTCCGCCT
 TCAATCCCAC CCGCTAAAGT ACTTGGAGCG GTCTCTCCCT CCCTCATCAG CCCACCAAAC
 CAAACCTAGC CTCCAAGAGT GGGGAAGAAAT TAAAGCAAGA TAGGCTATTA AGTGCAGAGG
 GAGAGAAAAT GCCTCCAACA TGTGAGGAAG TAATGAGAGA AATCATAGAA TTTCTTCCGC
 5 TTCCTCGCTC ACTGACTCGC TGCCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA
 CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG
 AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA
 TAGGCTCCGC CCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA
 CCCGACAGGA CTATAAGAT ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TGCCTCTCC
 10 TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCTTT CTCCCTTCGG GAAGCGTGGC
 GCTTTCTCAT AGCTCAGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTT GCTCCAAGCT
 GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACATCG
 TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG
 GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA
 15 CGGCTACACT AGAAGAACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG
 AAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT
 TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT
 TTCTACGGGG TCTGACGCTC AGTGAACGA AAATCAGCT TAAGGGATTT TGGTCATGAG
 ATTATCAAAA AGGATCTTCA CCTAGATCCT TTAAATTAA AAATGAAGTT TTAAATCAAT
 20 CTAAAGTATA TATGAGTAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC
 TATCTCAGCG ATCTGTCTAT TTCGTTTCAT CATAGTTGCC TGAATCGGG GGGGGGGGCG
 CTGAGGTCTG CCTCGTGAAG AAGGTGTTGC TGAATCATA CAGGCCTGAA TCGCCCCATC
 ATCCAGCCAG AAAGTGAGGG AGCCACGGTT GATGAGAGCT TTGTTGTAGG TGGACCAGTT
 GGTGATTTTG AACTTTTGCT TTGCCACGGA ACGGTCTGCG TTGTCGGGAA GATGCGTGAT
 25 CTGATCCTTC AACTCAGCAA AAGTTCGATT TATTCAACAA AGCCGCCGTC CCGTCAAGTC
 AGCGTAATGC TCTGCCAGTG TTACAACCAA TTAACCAATT CTGATTAGAA AAATCATCG
 AGCATCAAAT GAAACTGCAA TTTATTCTA TCAGGATTAT CAATACCATA TTTTGA
 AGCCGTTTCT GTAATGAAGG AGAAAACCTA CCGAGGCAGT TCCATAGGAT GGCAAGATCC
 TGGTATCGGT CTGCGATTCC GACTCGTCCA ACATCAATAC AACCTATTAA TTTCCCTCG
 30 TCAAAAATAA GGTATCAAG TGAGAAATCA CCATGAGTGA CGACTGAATC CCGTGAGAAT
 GGCAAAAGCT TATGCATTTT TTTCCAGACT TGTTCAACAG GCCAGCCATT ACGCTCGTCA
 TCAAAATCAC TCGCATCAAC CAAACCGTTA TTCATTCTG ATTGCGCCTG AGCGAGACGA
 AATACGCGAT CGCTGTTAAA AGGACAATTA CAAACAGGAA TCGAATGCAA CCGGCGCAGG
 AACACTGCCA GCGCATCAAC AATATTTTCA CCTGAATCAG GATATTCTTC TAATACCTGG

AATGCTGTTT TCCCGGGGAT CGCAGTGGTG AGTAACCATG CATCATCAGG AGTACGGATA
 AAATGCTTGA TGGTCGGAAG AGGCATAAAT TCCGTCAGCC AGTTTAGTCT GACCATCTCA
 TCTGTAACAT CATTTGGCAAC GCTACCTTTG CCATGTTTCA GAAACAAC TC TGGCGCATCG
 GGCTTCCCAT ACAATCGATA GATTGTCGCA CCTGATTGCC CGACATTATC GCGAGCCCAT
 5 TTATACCCAT ATAAATCAGC ATCCATGTTG GAATTTAATC GCGGCCCTCGA GCAAGACGTT
 TCCCGTTGAA TATGGCTCAT AACACCCCTT GTATTACTGT TTATGTAAGC AGACAGT TTT
 ATTGTTTCATG ATGATATATT TTTATCTTGT GCAATGTAAC ATCAGAGATT TTGAGACACA
 ACGTGGCTTT CCCCCCCCCC CCATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC
 GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC
 10 CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC CTATAAAAAT
 AGGCGTATCA CGAGGCCCTT TCGTC (SEQ ID NO:9) .

V1R – Vaccine vector V1R was constructed to obtain a minimum-sized vaccine vector without unneeded DNA sequences, which still retained the overall optimized heterologous gene expression characteristics and high plasmid yields that
 15 V1J and V1Jns afford. It was determined that (1) regions within the pUC backbone comprising the *E. coli* origin of replication could be removed without affecting plasmid yield from bacteria; (2) the 3'-region of the *kan^r* gene following the kanamycin open reading frame could be removed if a bacterial terminator was inserted in its place; and, (3) ~300 bp from the 3'- half of the BGH terminator could
 20 be removed without affecting its regulatory function (following the original KpnI restriction enzyme site within the BGH element). V1R was constructed by using PCR to synthesize three segments of DNA from V1Jns representing the CMVintA promoter/BGH terminator, origin of replication, and kanamycin resistance elements, respectively. Restriction enzymes unique for each segment were added to each
 25 segment end using the PCR oligomers: SspI and XhoI for CMVintA/BGH; EcoRV and BamHI for the *kan^r* gene; and, BclI and SalI for the *ori^r*. These enzyme sites were chosen because they allow directional ligation of each of the PCR-derived DNA segments with subsequent loss of each site: EcoRV and SspI leave blunt-ended DNAs which are compatible for ligation while BamHI and BclI leave complementary
 30 overhangs as do SalI and XhoI. After obtaining these segments by PCR each segment was digested with the appropriate restriction enzymes indicated above and then ligated together in a single reaction mixture containing all three DNA segments. The 5'-end of the *ori^r* was designed to include the T2 rho independent terminator sequence that is normally found in this region so that it could provide termination

information for the kanamycin resistance gene. The ligated product was confirmed by restriction enzyme digestion (>8 enzymes) as well as by DNA sequencing of the ligation junctions. DNA plasmid yields and heterologous expression using viral genes within V1R appear similar to V1Jns. The net reduction in vector size achieved was 1346 bp (V1Jns = 4.86 kb; V1R = 3.52 kb). PCR oligomer sequences used to synthesize V1R (restriction enzyme sites are underlined and identified in brackets following sequence) are as follows: (1) 5'-GGTACAAAATATTGGCTATTGGCCATTGCATACG-3' (SEQ ID NO:19) [SspI]; (2) 5'-CCACATCTCGAGGAACCGGGTCAATTCTTCAGCACC-3' (SEQ ID NO:20) [XhoI] (for CMVintA/BGH segment); (3) 5'-GGTACAGATATCGGAAAGCCACGTTGTG TCTCAAAATC-3' (SEQ ID NO:21) [EcoRV]; (4) 5'-CACATGGATCCGTAAT GCTCTGCCAGTGTTACAACC-3' (SEQ ID NO:2) [BamHI], (for kanamycin resistance gene segment) (5) 5'-GGTACATGATCACGTAGAAAAGATCA AAGGATCTTCTTG-3' (SEQ ID NO:23) [BclI]; (6) 5'-CCACATGTCGACCCGTAAA AAGGCCGCGTTGCTGG-3' (SEQ ID NO:24): [SalI], (for *E. coli* origin of replication).

The nucleotide sequence of vector V1R is as follows:

TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
20 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG
CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT AATCAATTAC
GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG
CCCGCCTGGC TGACCGCCCA ACGACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC
25 CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC
TGCCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC
TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
30 CGTCAATGGG AGTTTGT TTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGACGG TGGGAGGTCT ATATAAGCAG
AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA
TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA CGGTGCATTG GAACGCGGAT
TCCCCGTGCC AAGAGTGACG TAAGTACCGC CTATAGAGTC TATAGGCCCA CCCCCTTGGC

TTCTTATGCA TGCTATACTG TTTTGGCTT GGGGTCTATA CACCCCCGCT TCCTCATGTT
ATAGGTGATG GTATAGCTTA GCCTATAGGT GTGGGTATT GACCATTATT GACCACTCCC
CTATTGGTGA CGATACTTTC CATTACTAAT CCATAACATG GCTCTTTGCC ACAACTCTCT
TTATTGGCTA TATGCCAATA CACTGTCCTT CAGAGACTGA CACGGACTCT GTATTTTTAC
5 AGGATGGGGT CTCATTTATT ATTTACAAAT TCACATATAC AACACCACCG TCCCCAGTGC
CCGCAGTTTT TATTAAACAT AACGTGGGAT CTCCACGCGA ATCTCGGGTA CGTGTTCGGG
ACATGGGCTC TTCTCCGGTA GCGGCGGAGC TTCTACATCC GAGCCCTGCT CCCATGCCTC
CAGCGACTCA TGGTCGCTCG GCAGCTCCTT GCTCCTAACA GTGGAGGCCA GACTTAGGCA
CAGCAGATG CCCACCACCA CCAGTGTGCC GCACAAGGCC GTGGCGGTAG GGTATGTGTC
10 TGAAAATGAG CTCGGGGAGC GGGCTTGAC CGCTGACGCA TTTGGAAGAC TTAAGGCAGC
GGCAGAAGAA GATGCAGGCA GCTGAGTTGT TGTGTTCTGA TAAGAGTCAG AGGTAATCC
CGTTGCGGTG CTGTTAACGG TGGAGGGCAG TGTAGTCTGA GCAGTACTCG TTGCTGCCGC
GCGCGCCACC AGACATAATA GCTGACAGAC TAACAGACTG TTCCTTTCCA TGGGTCTTTT
CTGCAGTCAC CGTCCTTAGA TCTGCTGTGC CTTCTAGTTG CCAGCCATCT GTTGTTCGCC
15 CCTCCCCGT GCCTTCCTTG ACCCTGGAAG GTGCCACTCC CACTGTCCTT TCCTAATAAA
ATGAGGAAAT TGCATCGCAT TGTCTGAGTA GGTGTCATT CATTCTGGGG GGTGGGGTGG
GGCAGCACAG CAAGGGGGAG GATTGGGAAG ACAATAGCAG GCATGCTGGG GATGCGGTGG
GCTCTATGGG TACCCAGGTG CTGAAGAATT GACCCGGTTC CTCCTGGGCC AGAAAGAAGC
AGGCACATCC CCTTCTCTGT GACACACCCT GTCCACGCCC CTGGTTCTTA GTTCCAGCCC
20 CACTCATAGG AACTCATAG CTCAGGAGGG CTCCGCCTTC AATCCCACCC GCTAAAGTAC
TTGGAGCGGT CTCTCCCTCC CTCATCAGCC CACCAAACCA AACCTAGCCT CCAAGAGTGG
GAAGAAATTA AAGCAAGATA GGCTATTAAG TGCAGAGGGA GAGAAAATGC CTCCAACATG
TGAGGAAGTA ATGAGAGAAA TCATAGAATT TCTTCCGCTT CCTCGCTCAC TGA CTGCTG
CGCTCGGTCC TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA
25 TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAGGCC
AGGAACCGTA AAAAGGCCGC GTTGTGGCG TTTTCCATA GGCTCCGCC CCCTGACGAG
CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC
CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT GCCGCTTACC
GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCAATG CTCACGCTGT
30 AGGTATCTCA GTTCGGTGTA GGTCGTTGCG TCCAAGCTGG GCTGTGTGCA CGAACCCCCC
GTTACAGCCC ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGGTAAGA
CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC GAGGTATGTA
GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG AAGGACAGTA
TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA

TCCGGCAAAC AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACG
 CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC TGACGCTCAG
 TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG GATCTTCACC
 TAGATCCTTT TAAATTAAAA ATGAAGTTT AAATCAATCT AAAGTATATA TGAGTAAACT
 5 TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT
 CGTTCATCCA TAGTTGCCTG ACTCCGGGGG GGGGGGGCGC TGAGGTCTGC CTCGTGAAGA
 AGGTGTTGCT GACTCATACC AGGCCTGAAT CGCCCCATCA TCCAGCCAGA AAGTGAGGGA
 GCCACGGTTG ATGAGAGCTT TGTTGTAGGT GGACCAGTTG GTGATTTTGA ACTTTTGCTT
 TGCCACGGAA CCGTCTGCGT TGTCGGGAAG ATGCGTGATC TGATCCTTCA ACTCAGCAAA
 10 AGTTTCGATTT ATTCAACAAA GCCGCCGTCC CGTCAAGTCA GCGTAATGCT CTGCCAGTGT
 TACAACCAAT TAACCAATTC TGATTAGAAA AACTCATCGA GCATCAAATG AACTGCAAT
 TTATTCATAT CAGGATTATC AATACCATAT TTTTGAAAAA GCCGTTTCTG TAATGAAGGA
 GAAAACTCAC CGAGGCAGTT CCATAGGATG GCAAGATCCT GGTATCGGTC TGCGATTCCG
 ACTCGTCCAA CATCAATACA ACCTATTAAT TTCCCCTCGT CAAAAATAAG GTTATCAAGT
 15 GAGAAATCAC CATGAGTGAC GACTGAATCC GGTGAGAATG GCAAAAGCTT ATGCATTTCT
 TTCCAGACTT GTTCAACAGG CCAGCCATTA CGCTCGTCAT CAAAATCACT CGCATCAACC
 AAACCGTTAT TCATTCTGTA TTGCGCCTGA GCGAGACGAA ATACGCGATC GCTGTTAAAA
 GGACAATTAC AAACAGGAAT CGAATGCAAC CGGCGCAGGA AACTGCCAG CGCATCAACA
 ATATTTTCAC CTGAATCAGG ATATTCTTCT AATACCTGGA ATGCTGTTTT CCCGGGGATC
 20 GCAGTGGTGA GTAACCATGC ATCATCAGGA GTACGGATAA AATGCTTGAT GGTCGGAAGA
 GGCATAAAAT CCGTCAGCCA GTTTAGTCTG ACCATCTCAT CTGTAACATC ATTGGCAACG
 CTACCTTTGC CATGTTTCAG AAACAAC'TCT GCGCATCGG GCTTCCCATA CAATCGATAG
 ATTGTCGCAC CTGATTGCCC GACATTATCG CGAGCCCATT TATACCCATA TAAATCAGCA
 TCCATGTTGG AATTTAATCG CGGCCTCGAG CAAGACGTTT CCCGTTGAAT ATGGCTCATA
 25 ACACCCCTTG TATTACTGTT TATGTAAGCA GACAGTTTTA TTGTTTCATGA TGATATATTT
 TTATCTTGTG CAATGTAACA TCAGAGATTT TGAGACACAA CGTGGCTTTC CCCCCCCCCC
 CATTATTGAA GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT
 TAGAAAAATA AACAAATAGG GGTTCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC
 TAAGAAACCA TTATTATCAT GACATTAACC TATAAAAATA GGCGTATCAC GAGGCCCTTT
 30 CGTC (SEQ ID NO:25).

EXAMPLE 2

Codon Optimized HIV-1 Pol and HIV-1 IA Pol Derivatives as DNA Vector Vaccines

Synthesis of WT-optpol and IA-opt-pol Gene - Construction of both genes were

conducted by Midland Certified Reagent Company (Midland, TX) following

- 5 established strategies. Ten double stranded oligonucleotides, ranging from 159 to 340 bases long and encompassing the entire pol gene, were synthesized by solid state methods and cloned separately into pUC18. For the wt-pol gene, the fragments are as follows:

- | | | |
|----|--|-------------|
| | <i>Bgl</i> II#1- <i>Ecl</i> 136II half site at 282 | = pJS6A1-7 |
| 10 | <i>Pml</i> II half site at #285 - <i>Ecl</i> 136II half site at #597 | = pJS6B2-5 |
| | <i>Ssp</i> I half site at #600 - <i>Ecl</i> 136II half site at #866 | = pJS6C1-4 |
| | <i>Sma</i> I half site at #869 - <i>Apa</i> I #1095 | = pJS6D1-4 |
| | <i>Apa</i> I #1095 - <i>Kpn</i> I #1296 | = pJS6E1-4 |
| | <i>Kpn</i> I #1296 - <i>Xcm</i> I #1636 | = pJS6F1-5 |
| 15 | <i>Xcm</i> I #1636 - <i>Nsi</i> II #1847 | = pJS6G1-2 |
| | <i>Nsi</i> II #1847 - <i>Bcl</i> II half site at #2174 | = pJS6H1-14 |
| | <i>Bcl</i> II half site at #2174 - <i>Sac</i> I #2333 | = pJS6I1-2 |
| | <i>Sac</i> I #2333 - <i>Bgl</i> III #2577 | = pJS6J1-1 |

- 20 *Eco*RI and *Hind*III sequences were added upstream of each 5' end and downstream of each 3' end, respectively, to allow cloning into the *Eco*RI-*Hind*III sites of pUC18.

The next stage of the synthesis was to consolidate these cassettes into three roughly equal fragments (alpha, beta, gamma) and was performed as follows:

- 25 Alpha: The *Ssp*I-*Hind*III small fragment of pJS6C1-4 was transferred into the *Ecl*136II-*Hind*III sites of pJS6B2-5 to give pJS6BC1-1. Into the *Eco*RI-*Pml*II sites of this plasmid was inserted the *Eco*RI-*Ecl*136II small fragment of pJS6A1-7 to give pJS6 α 1-8.

- Beta: The *Eco*RI-*Apa*I small fragment of pJS6D1-4 was inserted into the corresponding sites of pJS6E1-2 to give pJS6DE1-2. Also, the *Eco*RI-*Xcm*I small fragment of pJS6F1-5 was inserted into the corresponding sites of pJS6G1-2 to give pJS6FG1-1. Then the *Eco*RI-*Kpn*I small fragment of pJS6DE1-2 was inserted into the corresponding sites of pJS6FG1-1 to give pJS6 β 1-1.

Gamma: The *Sac*I-*Hind*III small fragment of pJS6J1-1 was inserted into the corresponding sites of pJS6I1-2 to give pJS6IJ1-1. This plasmid was propagated through *E. coli* SCS110 (*dam*⁻/*dcm*⁻) to permit subsequent cleavage at the *Bcl*II site.

The *Bcl*II-*Hind*III small fragment of the unmethylated pJS6II1-1 was inserted into the *Bgl*II-*Hind*III sites of pJS6H1-14 to give pJS6 χ 1-1.

The wt-pol alpha, beta, gamma were ligated into the entire sequence as follows:

- 5 The *Eco*RI-*Ecl*136II small fragment of pJS6 α 1-8 was inserted into the *Eco*RI-*Sma*I sites of pJS6 β 1-1 to give pJS6 $\alpha\beta$ 2-1.

Into the *Nsi*II-*Hind*III sites of this plasmid was inserted the *Nsi*II-*Hind*III small fragment of pJS6 χ 1-1 to give pUC18-wt-pol. This final plasmid was completely resequenced in both strands.

- 10 To construct the entire IA-pol gene, only 3 new small fragments were synthesized:

*Pml*II half site at #285 – *Ecl*136II half site at #597 = pJS7B1-1

*Kpn*I #1296 – *Xcm*I #1636 = pJS7F1-2

*Nsi*II #1847 – *Bgl*II half site at #2174 = pJS7H1-5

- 15 These were then used in the same reconstruction strategy as described above to give pUC18-IA-pol.

- Expression Vector Construction* - pUC18-wt-pol and pUC18-IA-pol were digested with *Bgl*II in order to isolate fragments containing the entire pol genes. V1R, V1Jns, V1Jns-tpa (Shiver, et al., 1995, Immune responses to HIV gp120 elicited by DNA vaccination. In *Vaccines 95* (eds. Chanock, R. M., Brown, F., Ginsberg, H.S., & Norrby, E.) @ pp. 95-98; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; see also Example Section 1) were digested with *Bgl*II. The cut vectors were then treated with calf intestinal alkaline phosphatase. Both wt-pol and IA-pol genes were ligated into cut V1R using T4 DNA ligase (16 °C, overnight).
- 25 Competent DH5 α cells were transformed with aliquots of the ligation mixtures. Colonies were screened by restriction digestion of amplified plasmid isolates. Following a similar strategy, the *Bgl*II fragment containing the IA-pol was subcloned into the *Bgl*II site of V1Jns. To ligate the IA-pol gene into V1Jns-tpa, the IA-pol gene was PCR-amplified from V1R-IA-pol using pfu polymerase and the following pair of primers: 5'-GGTACAAGATCTCCGCCCCCATCTCCCCATTGAGA-3' (SEQ ID NO:26), and 5'-CCACATAGATCTGCCCGGGCTTTAGTCCTCATC-3' (SEQ ID NO:27). The upstream primer was designed to remove the initiation met codon and place the pol gene in frame with the tpa leader coding sequence from V1Jns-tpa. The PCR product was purified from the agarose gel slab using Sigma
- 30

DNA Purification spin columns. The purified products were digested with *Bgl*III and subcloned into the *Bgl*III site of V1Jns-tpa.

Results - The codon humanized wt- and IA-pol genes were constructed via stepwise ligation of 10 synthetic dsDNA fragments (Ferretti, et al., 1986, *Proc. Natl. Acad. Sci. USA* 83: 599-603). For expression in mammalian systems, the IA-pol gene was subcloned into V1R, V1Jns, and V1Jns-tpa. All these vectors place the gene under the control of the human cytomegalovirus/intron A hybrid promoter (hCMVIA). The DNA sequence of the IA-pol gene and the expressed protein product are shown in Figure 2A-B. Subcloning into V1Jns-tpa attaches the leader sequence from human tissue-specific plasminogen activator (tpa) to the N-terminus of the IA-pol (Pennica, et al., 1983, *Nature* 301: 214-221) to allow secretion of the protein. The sequences of the tpa leader and the fusion junction are shown in Figure 3.

EXAMPLE 3

15 HIV-1 POL Vaccine - Rodent Studies

Materials - *E. coli* DH5 α strain, penicillin, streptomycin, ACK lysis buffer, hepes, L-glutamine, RPMI1640, and ultrapure CsCl were obtained from Gibco/BRL (Grand Island, NY). Fetal bovine serum (FBS) was purchased from Hyclone. Kanamycin, Tween 20, bovine serum albumin, hydrogen peroxide (30%), concentrated sulfuric acid, β -mercaptoethanol (β -ME), and concanavalin A were obtained from Sigma (St. Louis, MO). Female balb/c mice at 4-6 wks of age were obtained from Taconic Farms (Germantown, NY). 0.3-mL insulin syringes were purchased from Myoderm. 96-well flat bottomed Maxisorp plates were obtained from NUNC (Rochester, NY). HIV-1_{IIIB} RT p66 recombinant protein was obtained from Advanced Biotechnologies, Inc. (Columbia, MD). 20-mer peptides were synthesized by Research Genetics (Huntsville, AL). Horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG1 was obtained from ZYMED (San Francisco, CA). 1,2-phenylenediamine dihydrochloride (OPD) tablets was obtained from DAKO (Norway). Purified rat anti-mouse IFN-gamma (IgG1, clone R4-6A2), biotin-conjugated rat anti-mouse IFN-gamma (IgG1, clone XMG 1.2), and streptavidin-alkaline phosphatase conjugate were purchased from PharMingen (San Diego, CA). 1-STEP NBT/BCIP dye was obtained from Pierce Chemicals (Rockford, IL). 96-well Multiscreen membrane plate was purchased from Millipore (France). Cell strainer was obtained from Becton-Dickinson (Franklin Lakes, NJ).

Plasmid Preparation - *E. coli* DH5 α cells expressing the pol plasmids were grown to saturation in LB broth supplemented with 100 μ g/mL kanamycin. Plasmid were purified by standard CsCl method and solubilized in saline at concentrations greater than 5 mg/mL until further use.

- 5 *Vaccination* - The plasmids were prepared in phosphate-buffered saline and administered into balb/c by needle injection (28-1/2G insulin syringe) of 50 μ L aliquot into each quad muscle. V1Jns-IApol was administered at 0.3, 3, 30 μ g dose and for comparison, V1Jns-tpa-IApol was given at 30 μ g dose. Immunizations were conducted at T=0 and T=8 wks (for select animals from the 30- μ g dose cohorts).
- 10 *ELISA Assay* - At T=12 wks, blood samples were collected by making an incision of a tail vein and the serum separated. Anti-RT titers were obtained following standard secondary antibody-based ELISA. Briefly, Maxisorp plates were coated by overnight incubation with 100 μ L of 1 μ g/mL HIV-1 RT protein (in PBS). The plates were washed with PBS/0.05% Tween 20 and incubated for approx. 2h with
- 15 200 μ L/well of blocking solution (PBS/0.05% tween/1% BSA). The blocking solution was decanted; 100 μ L aliquot of serially diluted serum samples were added per well and incubated for 2 h at room temperature. The plates were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L OPD/H₂O₂ solution for
- 20 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO₄ per well. OD₄₉₂ readings were recorded.

- ELIspot* - Spleens were collected from 5 mice/cohort at T=13-14 wks and pooled into a tube of 8-mL R10 medium (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 μ M β -ME).
- 25 Multiscreen opaque plates were coated with 100 μ L/well of capture mAb (purified R4-6A2 diluted in PBS to 5 μ g/ml) at 4°C overnight. The plates were washed with PBS/Pen/Strep in hood and blocked with 200 μ L/well of complete R10 medium for 37°C for at least 2 hrs. The mouse spleens were ground on steel mesh, collected into 15ml tubes and centrifuged at 1200rpm for 10min. The pellet was treated in ACK
- 30 buffer (4ml of lysis buffer per spleen) for 5min at room temperature to lyse red blood cells. The cell pellet was centrifuged as before, resuspended in K-medium (5ml per mouse spleen), filtered through a cell strainer and counted using a hemacytometer. Block medium was decanted from the plates and 100 μ L/well of cell samples (5.0x10⁵ cells per well) plus antigens were added. Pol-specific CD4⁺ cells were stimulated

- using a mixture of previously identified two epitope-containing peptides (aa641-660, aa731-750). Antigen-specific CD8+ cells were stimulated using a pool of four peptide epitope-containing peptides (aa201-220, aa311-330, aa571-590, aa781-800) or with individual peptides. A final concentration of 4 ug/mL per peptide was used.
- 5 Each splenocyte sample is tested for IFN-gamma secretion by adding the mitogen, concanavalin A. Plates were incubated at 37°C, 5% CO₂ for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and soaked with 100 uL/well of 5 ug/mL biotin-conjugated rat anti-mouse IFN- mAb (clone XMG1.2) at 4°C overnight. The plates were washed and soaked with 100 uL/well 1/2500 dilution of streptavidin-AP
- 10 (in PBS/0.005% Tween/5%FCS) for 30 min at 37 °C. Following a wash, spots were developed by incubating with 100µl/well 1-step NBT/BCIP for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each wells were determined using a dissecting microscope and normalized to 10e6 cells.

- Results* - Single vaccination of balb/c mice with V1Jns-IApol is able to induce
- 15 antigen-specific antibody (Figure 4) and T cell (Figure 5) responses in a dose response manner. IFN-gamma secretion from splenocytes can be detected from 3 and 30 ug cohort following stimulation with pools of peptides that contain CD4+ and CD8+ T cell epitopes. These epitopes were identified by (1) screening 20-mer peptides that encompass the entire pol sequence and overlap by 10 amino acid for
- 20 ability to stimulate IFN-gamma secretion from vaccinee splenocytes, and (2) determining the T cell type (CD4+ or CD8+) by depleting either population in an Elispot-assay. Addition of tpa leader sequence to the pol gene is able to induce comparable, if not slightly higher, frequencies of pol-specific CD4+ and CD8+ cells. A second immunization with either V1Jns-IApol and V1Jns-tpa-IApol resulted in
- 25 effective boosting of the immune responses.

EXAMPLE 4

HIV-1 Pol Vaccine - Non Human Primate Studies

- Materials* - *E. coli* DH5α strain, penicillin, streptomycin, and ultrapure CsCl
- 30 were obtained from Gibco/BRL (Grand Island, NY). Kanamycin and phytohemagglutinin (PHA-M) were obtained from Sigma (St. Louis, MO). 20-mer peptides were synthesized by SynPep (Dublin, CA) and Research Genetics (Huntsville, AL). 96-well Multiscreen Immobilon-P membrane plates were obtained from Millipore (France). Streptavidin-alkaline phosphatase conjugate were purchased

form Pharmingen (San Diego, CA). 1-Step NBT/BCIP dye was obtained from Pierce Chemicals (Rockford, IL). Rat anti-human IFN-gamma mAb and biotin-conjugated anti-human IFN-gamma reagent were obtained from R&D Systems (Minneapolis, MN). Dynabeads M-450 anti-human CD4 were obtained from Dynal (Norway).

- 5 HIVp24 antigen assay was purchased from Coulter Corporation (Miami, FL). HIV-1_{IIIIB} RT p66 recombinant protein was obtained from Advanced Biotechnologies, Inc. (Columbia, MD). Plastic 8 well strips/plates, flat bottom, Maxisorp, are obtained from NUNC (Rochester, NY). HIV+ human serum 9711234 was obtained from Biological Specialty Corp.

- 10 *Plasmid Preparation* - *E. coli* DH5 α cells expressing the pol plasmids were grown to saturation in LB supplemented with 100 ug/mL kanamycin. Plasmid were purified by standard CsCl method and solubilized in saline at concentrations greater than 5 mg/mL until further use.

- Vaccination* - Cohorts of 3 rhesus macaques (approx. 5-10 kg) were
15 vaccinated with 5 mg dose of either V1Jns-IApol or V1Jns-tpa-IApol. The vaccine was administered by needle injection of two 0.5 mL aliquots of 5 mg/mL plasmid solution (in phosphate-buffered saline, pH 7.2) into both deltoid muscles. Prior to vaccination, the monkeys were chemically restraint with i.m. injection of 10 mg/kg ketamine. The animals were immunized 3x at 4 week intervals (T=0, 4, 8 wks).

- 20 *Sample Collection* - Blood samples were collected at T = 0, 4, 8, 12, 16, 18 wks; sera and PBMCs were isolated using established protocols.

- ELISpot Assay* - Immobilon-IP plates were coated with 100 uL/well of rat anti-human IFN-gamma mAb at 15 ug/mL at 4 °C overnight. The plates are then washed with PBS and block by adding 200 uL/well of R10 medium. 4x10⁵ peripheral blood
25 cells were plated per well and to each well, either media or one of the pol peptide pools (final concentration of 4 ug/mL per peptide) or PHA, a known mitogen, is added to a final volume of 100 uL. Duplicate wells were set up per sample per antigen and stimulation was performed for 20-24 h at 37 °C. The plates are then washed; biotinylated anti-human IFN-gamma reagent is added (0.1 ug/mL, 100 uL
30 per well) and allowed to incubate for overnight at 4 °C. The plates are again washed and 100 uL of 1:2500 dilution of the strepavidin-alkaline phosphatase reagent (in PBS/0.005% Tween/5% FCS) is added and allowed to incubate for 2 h at ambient room temperature. After another wash, spots are developed by incubating with 100 uL/well of 1-step NBT/BCIP for 6-10 min. CD4- T cell depletion was performed by

adding 1 bead particle/10 cell of Dynabeads M450 anti-human CD4, prewashed with PBS, and incubating on the shaker at 4 °C for 30 min. The beads are fractionated magnetically and the unbound cells collected and quantified before plating onto the ELISpot assay plates (at 4x10e5 cells per well).

- 5 *CTL Assay* - Procedures for establishing bulk CTL culture with fresh or cryopreserved peripheral blood mononuclear cells (PBMC) are as follows. Twenty percent total PBMC were infected in 0.5 ml volume with recombinant vaccinia virus, Vac-tpaPol, respectively, at multiplicity of infection (moi) of 5 for 1 hr at 37°C, and then combined with the remaining PBMC sample. The cells were washed once in 10
10 ml R-10 medium, and plated in a 12 well plate at approximately 5 to 10 x 10⁶ cells/well in 4 ml R-10 medium. Recombinant human IL-7 was added to the culture at the concentration of 330 U/ml. Two or three days later, one milliliter of R-10 containing recombinant human IL-2 (100 U/ml) was added to each well. And twice weekly thereafter, two milliliters of cultured media were replaced with 2 ml fresh R-
15 10 medium with rhIL-2 (100 U/ml). The lymphocytes were cultured at 37°C in the presence of 5% CO₂ for approximately 2 weeks, and used in cytotoxicity assay as described below. The effector cells harvested from bulk CTL cultures were tested against autologous B lymphoid cell lines (BLCL) sensitized with peptide pools. To prepare for the peptide-sensitized targets, the BLCL cells were washed once with
20 R-10 medium, enumerated, and pulsed with peptide pool (about 4 to 8 µg/ml concentration for each individual peptide) in 1 ml volume overnight. A mock target was prepared by pulsing cells with peptide-free DMSO diluent to match the DMSO concentration in the peptide-pulsed targets. The cells were enumerated the next morning, and 1 x 10⁶ cells were resuspended in 0.5 ml R-10 medium. Five to ten
25 microliters of Na⁵¹CrO₄ were added to the tubes at the same time, and the cells were incubated for 1 to 2 hr 37°C. The cells were then washed 3 times and resuspended at 5x10⁴ cells/ml in R-10 medium to be used as target cells. The cultured lymphocytes were plated with target cells at designated effector to target (E:T) ratios in triplicates in 96-well plates, and incubated at 37°C for 4 hours in the presence of 5% CO₂. A
30 sample of 30 µl supernatant from each well of cell mixture was harvested onto a well of a Lumaplate-96 (Packard Instrument, Meriden, CT), and the plate was allowed to air dry overnight. The amount of ⁵¹Cr in the well was determined through beta-particle emission, using a plate counter from Packard Instrument. The percentage of specific lysis was calculated using the formula as: % specific lysis = (E-S) / (M-S).

The symbol E represents the average cpm released from target cells in the presence of effector cells, S is the spontaneous cpm released in the presence of medium only, and M is the maximum cpm released in the presence of 2% Triton X-100.

ELISA Assay - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Repeated vaccinations with V1Jns-IApol induced in 1 of 3 monkeys (94R033) significant levels of antigen-specific T cell activation (Figure 6A-C and Table 2) and CTL killing of peptide-pulsed autologous cells (Figure 7A-B). A significant CD8+ component to the T cell responses in this animal was confirmed by peptide-stimulation of CD4-depleted PBMCs in an ELISPOT assay (Table 2).

Immunization with V1Jns-tpa-IApol produced T cell responses from all 3 vaccinees (Figures 6A-C, Figure 7A-B; Table 2). Two (920078, 94R028) exhibited bulk CTL activity and detectable CD8+ components as measured by Elispot analyses of CD4-depleted PBMCs. For the third monkey (920073), the activated T cells were largely CD4+ (Table 2). Table 3 shows the time course data on the frequency of IFN-gamma secreting cells (SFC/million cells) upon antigen-specific stimulation for monkeys vaccinated 3x with either V1Jns-IApol or V1Jns-tpa-IApol (5 mg dose). At T=18 wks, CD4-cell depletion were performed; the reported values are the number of spots per million of fractionated cells and are not corrected for the resultant enrichment of CD8+ T cells. PBMCs were stimulated with peptide pools that represent either IA pol protein (mpol-1, mpol-2) or wt Pol (wtpol-1, wtpol-2).

TABLE 2

Vaccine	Animal No.	Antigen	T=0 wk	T=4 Wk	T=8 Wk	T=12 Wk	T=18 Wk	
			Dose 1	Dose 2	Dose 3			CD4-Depd
VLJns-IAPd 5 mgs	94R008	medium	1	15	6	11	11	11
		mpd-1	3	69	28	61	20	15
		mpd-2	0	25	21	19	28	16
		wtpd-1		49	20	53	18	
		wtpd-2		34	24	24	19	
	94R013	medium	0	14	6	9	18	11
		mpd-1	0	9	63	25	34	9
		mpd-2	1	15	24	36	24	15
		wtpd-1		9	50	33	18	
		wtpd-2		6	21	29	25	
	94R033	medium	4	15	11	14	13	8
		mpd-1	3	29	86	51	41	24
		mpd-2	0	24	25	43	59	64
		wtpd-1		30	38	60	53	
		wtpd-2		48	46	86	61	
VLJns-tpd-IAPd 5 mgs	920078	medium	0	24	13	11	14	11
		mpd-1	3	110	120	119	155	11
		mpd-2	1	221	130	561	289	145
		wtpd-1		115	53	70	116	
		wtpd-2		218	204	490	194	
	920073	medium	0	13	3	15	15	6
		mpd-1	0	36	51	113	90	14
		mpd-2	0	29	16	83	115	34
		wtpd-1		20	35	100	74	
		wtpd-2		25	16	79	61	
	94R028	medium	0	18	11	18	19	9
		mpd-1	1	30	24	29	30	28
		mpd-2	1	24	23	66	59	95
		wtpd-1		23	25	34	29	
		wtpd-2		26	28	71	40	
Naïve	920072	medium	1	19	3	38	9	4
		mpd-1	0	24	11	25	4	6
		mpd-2	1	24	5	28	6	5
		wtpd-1		18	13	20	6	
		wtpd-2		23	14	33	14	

For the Elispot assay, antigen specific stimulation were performed by using pools of 20-mer peptide pools based on the vaccine sequence. The vaccine pol sequence differs from the wild-type HIV-1 sequence by 9 point mutations, thereby affecting 16 of the 20-mer peptides in the pool. Comparable responses were observed in the vaccinees when these peptides are replaced with those using the wild-type sequences.

Four of the vaccinees gave anti-RT titers above background after 3 dosages of the plasmids (Table 2).

TABLE 3

Anti-RT levels in Rhesus Macaques Vaccinated 3x (4 week intervals) with 5 mgs of V1Jns-IApol or V1Jns-tpa-IApol expressed in mMU/mL.

Vaccine/Monkey	T=0Wk	T=4	T=8	T=12	T=16
	DOSE 1	DOSE 2	DOSE 3		
V1Jns-IApol, 5 mg					
94R008	ND	<10	<10	15	14
94R013	ND	<10	<10	<10	<10
94R033	ND	<10	<10	25	19
V1Jns-tpa-IApol, 5 mg					
920078	ND	<10	<10	35	17
920073	ND	<10	<10	<10	<10
94R028	ND	<10	<10	20	63

EXAMPLE 5

Effect of Codon Optimization on In Vivo Expression and
Cellular Immune Response of wt-pol

Materials and Methods - Extraction of virus-derived pol gene - The gene for RT-IN (wt-pol; a non-codon optimized wild type pol gene derived directly from the HIV IIIB genome) was extracted and amplified from the HIV IIIB genome using two primers, 5'-CAG GCG AGA TCT ACC ATG GCC CCC ATT AGC CCT ATT GAG ACT GTA-3' (SEQ ID NO:29) and 5'-CAG GCG AGA TCT GCC CGG GCT TTA ATC CTC ATC CTG TCT ACT TGC CAC-3' (SEQ ID NO:30), containing *Bgl*III sites.

The reaction contained 200 nmol of each primer, 2.5 U of pfu Turbo DNA polymerase (Stratagene, La Jolla, CA), 0.2 mM of each dNTPs, and the template DNA in 10mM KCl, 10mM (NH₄)₂SO₄, 20mM Tris-HCl pH 8.75, 2mM MgSO₄, 0.1% TritonX-100, 0.1mg/ml bovine serum albumin (BSA). Thermocycling

conditions were as follows: 20 cycles of 1 min at 95 °C, 1 min at 56 °C, and 4 mins at 72 °C with 15-min capping at 72 °C. The digested PCR fragment was subcloned into the *Bgl*III site of the expression plasmid V1Jns (Shiver, et al., 1995, Immune responses to HIV gp120 elicited by DNA vaccination. In Chanock, R. M., Brown, F., Ginsberg, H. S., and Norrby, E. (Eds.) *Vaccines 95*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 95-98; see also Example section 1 herein) expression plasmid following similar procedures as described above. The ligation mixtures were then used to transform competent *E. coli* DH5 cells and screened by PCR amplification of individual colonies. Sequence of the entire gene insert was confirmed. All plasmid constructs for animal immunization were purified by CsCl method (Sambrook, et al., 1989, Fritsch and Maniatis, T. (Eds) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor).

In vitro expression in mammalian cells - 1.5×10^6 293 cells were transfected with 1 or 10 µg of V1R-wt-pol (codon optimized) and V1Jns-wt-pol (virus derived) using the Cell Phect kit and incubated for 48 h at 37 °C, 5% CO₂, 90% humidity. Supernatants and cell lysates were prepared and assayed for protein content using Pierce Protein Assay reagent (Rockford, IL). Aliquots containing equal amounts of total protein were loaded onto 10-20% Tris glycine gel (Novex, San Diego, CA) along with the appropriate molecular weight markers. The pol product was detected using anti-serum from a seropositive patient (Scripps Clinic, San Diego, CA) diluted 1:1000 and the bands developed using goat anti-human IgG-HRP (Bethyl, Montgomery, TX) at 1:2000 dilution and standard ECL reagent kit (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Ultrasensitive RT activity assay of pol constructs - RT activities from codon optimized wt-pol and IA pol plasmids were analyzed by the Product-Enhanced Reverse Transcriptase (PERT) assay using Perkin Elmer 7700, Taqman technology (Arnold, et al., 1999, One-step fluorescent probe product-enhanced reverse transcriptase assay. In McClelland, M., Pardee, A. (Eds.) *Expression genetics: accelerated and high-throughput methods*. Biotechniques Books, Natick, MA, pp. 201-210). Background levels for this assay were determined using 1:100,000 dilution of lysates from mock (chemical treatment only, no vector) transfected 293 cells. This background range is set as RT/reaction tube of 0.00 to 56.28 which is taken from the mean value of 13.80 +/- 3 standard deviations (sd=14.16). Any individual value >56.28 would be considered positive for PERT assay. Cells lysates were prepared

similarly for the following samples: mock transfection with empty V1Jns vector; no vector control; transfection with V1Jns-tpa-pol (codon optimized); and transfection with V1Jns-IApol (codon optimized). Samples were serially diluted to 1:100,000 in PERT buffer and 24 replicates for each sample at this dilution were assayed for RT activity.

Rodent immunization with optimized and virus-derived pol plasmids - To compare the immunogenic properties of wt-pol (codon optimized) and virus-derived pol gene, cohorts of BALB/c mice (N=10) were vaccinated with 1 µg, 10 µg, and 100 µg doses of V1R-wt-pol (codon optimized) and V1Jns-wt-pol plasmid (virus derived).

At 5 weeks post dose 1, 5 of 10 mice per cohort were boosted with the same dose of plasmid they initially received. In all cases, the vaccines were suspended or diluted in 6 mM sodium phosphate, 150 mM sodium chloride, pH 7.2, and the total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Anti-RT ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 µL of 1 µg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for approximately 2h with 200 µL/well of blocking solution (PBS/0.05% tween/1% BSA). The blocking solution was decanted; 100 µL aliquot of serially diluted serum samples were added per well and incubated for 2 h at room temperature. An initial dilution of 100-fold is performed followed by 4-fold serial dilution. The plates were washed and 100 µL of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 µL 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 µL of 0.5M H₂SO₄ per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value):

ELISpot assay - Antigen-specific INF γ -secreting cells from mouse spleens were detected using the ELISpot assay (Miyahira, et al., 1995, Quantification of antigen specific CD8⁺ T cells using an ELISPOT assay. *J. Immunol. Methods* 1995,

181, 45-54). Typically, spleens were collected from 3-5 mice/cohort and pooled into a tube of 8-mL complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β -ME). Multiscreen opaque plates (Millipore, France) were coated with 100 μ L/well of 5 μ g/mL purified rat anti-mouse IFN- γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), in PBS at 4°C overnight. The plates were washed with PBS/penicillin/streptomycin in hood and blocked with 200 μ L/well of complete RPMI media for 37 °C for at least 2 h. The mouse spleens were ground on steel mesh, collected into 15ml tubes and centrifuged at 1200rpm for 10 min. The pellet was treated with 4 mL ACK buffer (Gibco/BRL) for 5 min at room temperature to lyse red blood cells. The cell pellet was centrifuged as before, resuspended in complete RPMI media (5 ml per mouse spleen), filtered through a cell strainer and counted using a hemacytometer. Block media was decanted from the plates and to each well, 100 μ L of cell samples (5×10^5 cells per well) and 100 μ L of the antigen solution were added. To the control well, 100 μ L of the media were added; for specific responses, peptide pools containing either CD4⁺ or CD8⁺ epitopes were added. In all cases, a final concentration of 4 μ g/mL per peptide was used. Each sample/antigen mixture were performed in triplicate wells. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μ L/well of 1.25 μ g/mL biotin-conjugated rat anti-mouse IFN- γ mAb, clone XMG1.2 (Pharmingen) at 4°C overnight. The plates were washed and incubated with 100 μ L/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Following a wash, spots were developed by incubating with 100 μ L/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10^6 cell input.

Results - In vitro expression of Pol in mammalian cells - Heterologous expression of the optimized wt or IA pol genes (V1R-wt-pol (codon optimized), V1Jns-IApol (codon optimized), V1Jns-tpa-IApol (codon optimized)) in 293 cells (Figure 8) yielded a single polypeptide of correct approximate molecular size (90-kDa) for the RT-IN fusion product. In contrast, no expression could be detected by transfecting cells with 1 and 10 μ g of the V1Jns-wt-pol, which bears the virus-derived *pol*.

Ultrasensitive RT assay of cells transfected with Pol constructs - Table 4 summarizes the levels of polymerase activity from mock (vector only) control, IAPol (codon optimized) and wt-pol plasmids (codon optimized). Results indicate that the wild-type POL transfected cells contained RT activity approximately 4-5 logs higher than the 293 cell only baseline values. Mock transfected cells contained activity no higher than baseline values. The RT activity from opt-IAPol-transfected cells was also found to be no different than baseline values; no individual reaction tube resulted in RT activity higher than the established cut-off value of 56.

10

Table 4

Sample	Avg. RT/tube	Standard deviation	Minimum	Maximum
Vector only	16.25	18.52	0.0	42.99
IAPol (codon optimized)	2.99	8.01	0.0	35.20
Wt-pol (codon optimized)	126147	21338	68973	152007

Comparative immunogenicity of optimized and virus-derived pol plasmid - To compare the *in vivo* potencies of both constructs, BALB/c mice (N=10 per group) were vaccinated with escalating doses (1, 10, 100 µg) of either V1Jns-wt-pol (virus derived) or V1R-wt-pol (codon optimized). At 5 wks post dose 1, 5 of 10 animals were randomly boosted with the same vaccine and dose they received initially. Figure 9 shows the geometric mean titers of the BALB/c cohorts determined at 2 wks past boost. No significant anti-RT titers can be observed from animals immunized with one or two doses of the wt-pol plasmid (virus derived). In contrast, animals vaccinated with the humanized gene construct gave cohort anti-RT titers (>1000) significantly above background levels at doses above 10 ug. The responses seen at 10 and 100 ug dose of V1R-wt-pol (codon optimized) were boosted approximately 10-fold with a second immunization, reaching titers as high as 10^6 . Splens from all mice in each of the cohorts were collected to be analyzed for IFN- γ secretion following stimulation with mixtures of either CD4+ peptide epitopes or CD8+ peptide epitopes. The results are shown in Figure 10. All wt-pol vaccinees did

not show any significant cellular response above the background controls. In contrast, strong antigen-stimulated IFN- γ secretion were observed in a dose-responsive manner from animals vaccinated with one or two doses of 10 or more μg of the wt-pol (codon optimized) construct.

- 5 The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

10

WHAT IS CLAIMED IS:

1. A pharmaceutically acceptable DNA vaccine composition, which comprises:
 - (a) a DNA expression vector; and,
 - 5 (b) a DNA molecule containing a codon optimized open reading frame encoding a Pol protein or inactivated Pol derivative thereof, wherein upon administration of the DNA vaccine to a host the Pol protein or inactivated Pol derivative is expressed and generates a cellular immune response against HIV-1 infection.
- 10 2. The DNA vaccine of claim 1 wherein the DNA molecule encodes wild type Pol.
3. The DNA vaccine of claim 2 wherein the DNA molecule comprises
15 the nucleotide sequence as set forth in SEQ ID NO:1.
4. The DNA vaccine of claim 3 which is V1Jns-wt-pol.
5. The DNA vaccine of claim 1 wherein the DNA molecule encodes an
20 inactivated Pol derivative which contains a nucleotide sequence encoding a human tissue plasminogen activator leader peptide.
6. The DNA vaccine of claim 5 wherein the DNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:5
25
7. The DNA vaccine of claim 6 which is V1Jns-tPA-wt-pol.
8. The DNA vaccine of claim 1 wherein the inactivated Pol protein contains at least one amino acid modification within each region of the Pol protein
30 responsible for reverse transcriptase activity, RNase H activity and integrase activity, such that the inactivated Pol protein shows no substantial reverse transcriptase activity, RNase H activity and integrase activity.

9. The DNA vaccine of claim 8 wherein the DNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:3

10. The DNA vaccine of claim 9 which is V1Jns-IAPol.

11. The DNA vaccine of claim 8 wherein the DNA molecule encodes an inactivated Pol derivative which contains a nucleotide sequence encoding a human tissue plasminogen activator leader peptide.

12. The DNA vaccine of claim 11 wherein the DNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:7.

13. The DNA vaccine of claim 7 which is V1Jns-tPA-IAPol.

14. A method for inducing an immune response against infection or disease caused by virulent strains of HIV which comprises administering into the tissue of a mammalian host a pharmaceutically acceptable DNA vaccine composition which comprises a DNA expression vector and a DNA molecule containing a codon optimized open reading frame encoding a Pol protein or inactivated Pol derivative thereof, wherein upon administration of the DNA vaccine to the vertebrate host the Pol protein or inactivated Pol derivative is expressed and generates the immune response.

15. The method of claim 16 wherein the mammalian host is a human.

16. The method of claim 17 wherein the DNA vaccine is selected from the group consisting of V1Jns-WTPol, V1Jns-tPA-WTPol, V1Jns-IAPol and V1Jns-tPA-IAPol.

17. A substantially purified protein which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

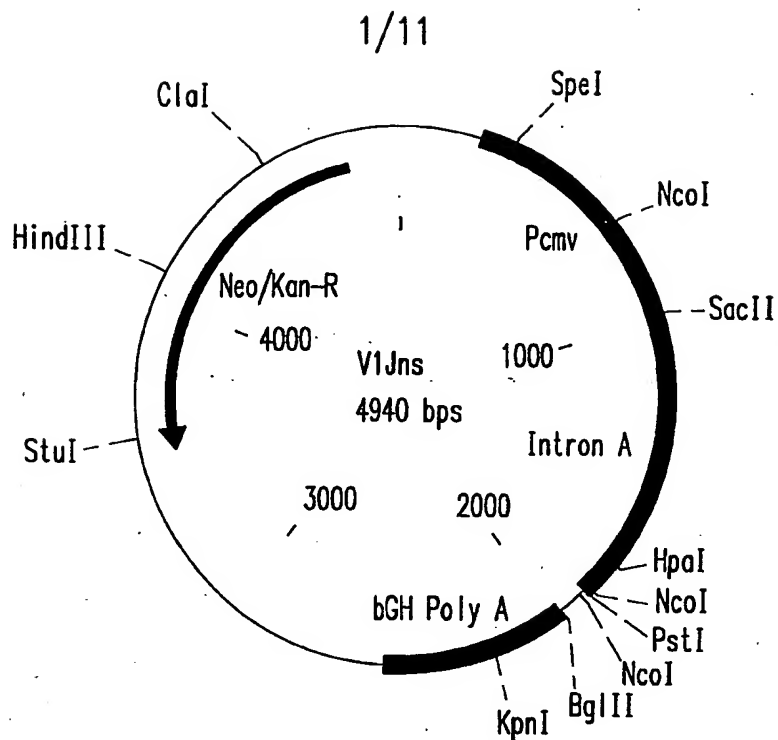


FIG. 1A

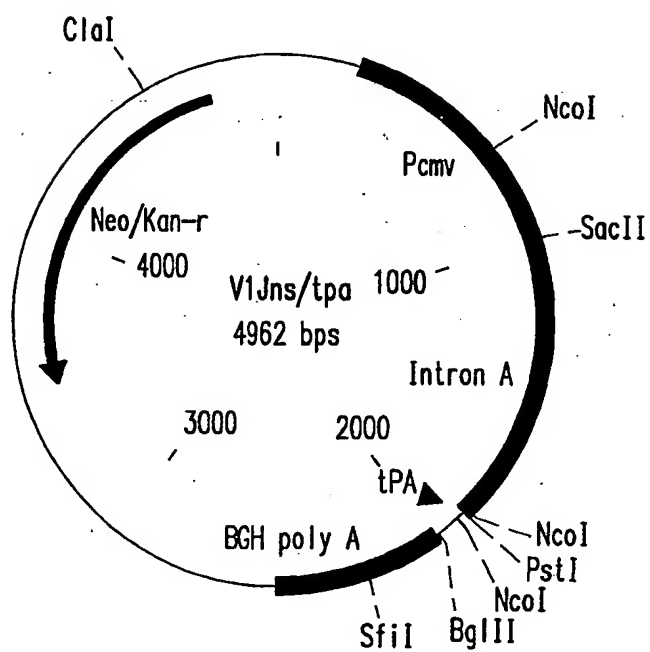


FIG. 1B

2/11

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Bg/II MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLys

1

10

20

GCAGTGGCCCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA
sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL

30

40

50

AGATTGCCCCGAGAACCCTACAACACCCTGTGTTTGCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG
ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal

60

70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA
AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLys

80

90

100

GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGATGCCTACTTCTCTGTGCCCCCTGGATGAGGACTTCAGGAAGTACACTG
sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA

110

120

130

CCTTCACCATCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCACTACAATGTGCTGCCCCAGGGCTGGAAGGGC
loPheThrIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly

140

150

TCCCTGCCATCTTCCAGTCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCCTGACATTGTGATCTACCA
SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGln

160

170

180

GTACATGGCTGCCCTGTATGTGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC
nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL

190

200

210

TGCTGAGGTGGGCCTGACCACCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC
euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis

220

230

CCCGACAAGTGGACTGTGCAGCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG
ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValGln

240

250

260

CAAGCTGAAGTGGGCCTCCCAATCTACCCTGGCATCAAGGTAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCC
yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL

270

280

290

FIG.2A

SUBSTITUTE SHEET (RULE 26)

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TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGGAGATCCTGAAGGAGCCTGTGCAT
EuThrGluValIleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProValHis
300 310

GGGGTGACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAATCTA
GlyValTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy
320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCACACCAATGATGTGAAGCAGCTGA
rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMetArgGlyAlaHisThrAsnAspValLysGlnLeuT
350 360 370

CTGAGGCTGTGCAGAAGATCACCCTGAGTCCATTGTGATCTGGGGCAAGACCCCAAGTTCAAGCTGCCCATCCAGAAG
hrGluAlaValGlnLysIleThrThrGluSerIleValIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys
380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCT
GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheValAsnThrProProLe
400 410 420

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATGTGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG
uValLysLeuTrpTyrGlnLeuGluLysGluProIleValGlyAlaGluThrPheTyrValAlaGlyAlaAlaAsnArgG
430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGGCAGGCAGAAGGTGGTGACCCTGACTGACACCACCAACCAG
luThrLysLeuGlyLysAlaGlyTyrValThrAsnArgGlyArgGlnLysValValThrLeuThrAspThrThrAsnGln
460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC
LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluValAsnIleValThrAlaSerGlnTyrAl
480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG
aLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuValAsnGlnIleIleGluGlnLeuIleLysLysG
510 520 530

AGAAGGTGTACCTGGCCTGGGTGCCTGCCCACAAGGGCATTGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
luLysValTyrLeuAlaTrpValProAlaHisLysGlyIleGlyGlyAsnGluGlnValAspLysLeuValSerAlaGly
540 550

ATCAGGAAGGTGCTGTTCTCTGGATGGCATTGACAAGGCCAGGATGAGCATGAGAAGTACCACTCCAAGTGGAGGGCTAT
IleArgLysValLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe
560 570 580

FIG.2B

SUBSTITUTE SHEET (RULE 26)

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GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCCTCCTGTGACAAGTCCAGCTGAAGGGGAGG
tAlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluA
590 600 610

CCATGCATGGCCAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCCTGCACCCACCTGGAGGCAAGGTGATCCTGGTG
laMetHisGlyGlnValAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysValIleLeuVal
620 630

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT
AlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe
640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACCTCACTGGGGCCACAGTGAGGGCTG
uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrValArgAlaA
670 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTGGCATCCCCTACAACCCCCAGTCCCAGGGGTGGTGGCCTCCATGAAC
laCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyValValAlaSerMetAsn
700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTTCAT
LysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIle
720 730 740

CCACAACCTTCAAGAGGAAGGGGGGCATCGGGGCTACTCCGCTGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleG
750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAGATCCAGAACCTCAGGGTGACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGGGGAGGGGGCTGTGGTGATCCAGGACAACCTGACATCAAGGTGGTGGCCAG
LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValValProAr
800 810 820

GAGGAAGGCCAAGATCATCAGGGACTATGGCAAGCAGATGGCTGGGGATGACTGTGTGGCCTCCAGGCAGGATGAGGACT
gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspX
830 840 850

AAAGCCCGGGCAGATCT (SEQ ID NO: 3)
Xx Bg/II

FIG.2C

SUBSTITUTE SHEET (RULE 26)

GATCACCATTGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGTGTCGACGAGTCTTCGTTTCGC
MetAspAlaMetLysArgGlyLeuCysCysValLeuleuCysGlyAlaValPheValSerP
-25 -20 -10

CCAGCGAGATCTCCGCCCCCATCTCCCCATTGAGACTGTGCCCTGTGAAGCTGAAGCCTCGCATGCGATGCC (within SEQ ID NO: 7)
RoSerGluIleSerAlaProIleSerProIleGluThrValProValLysLeulysProGlyMetAspGly (within SEQ ID NO: 8)
-1 2 10 20

FIG. 3

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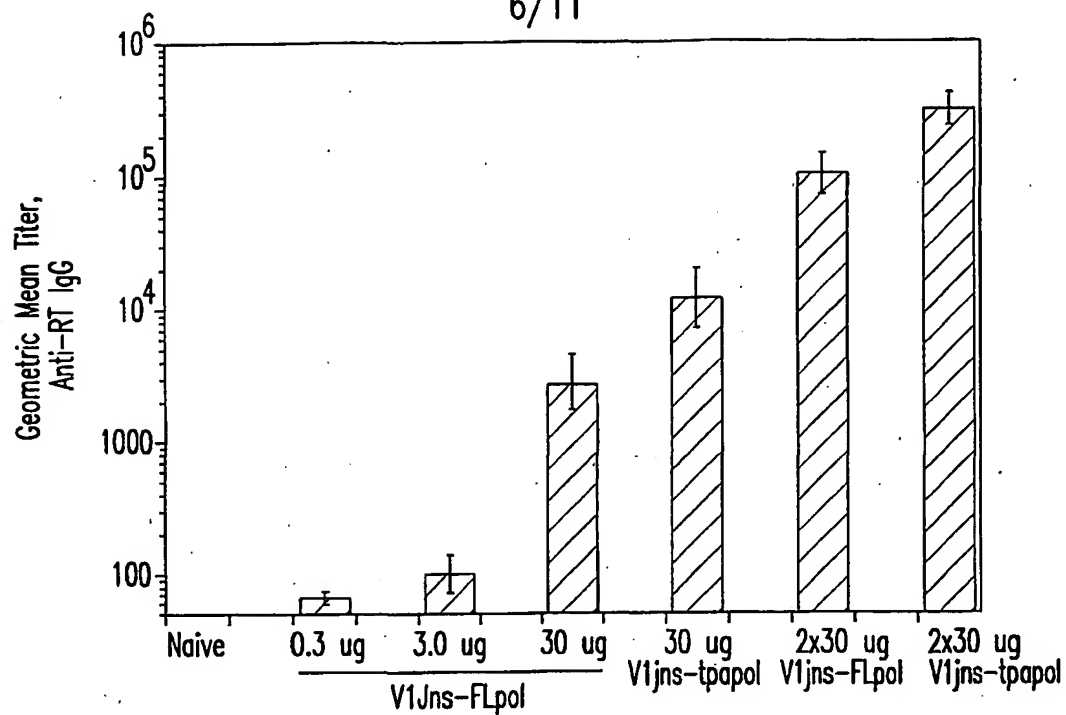


FIG.4

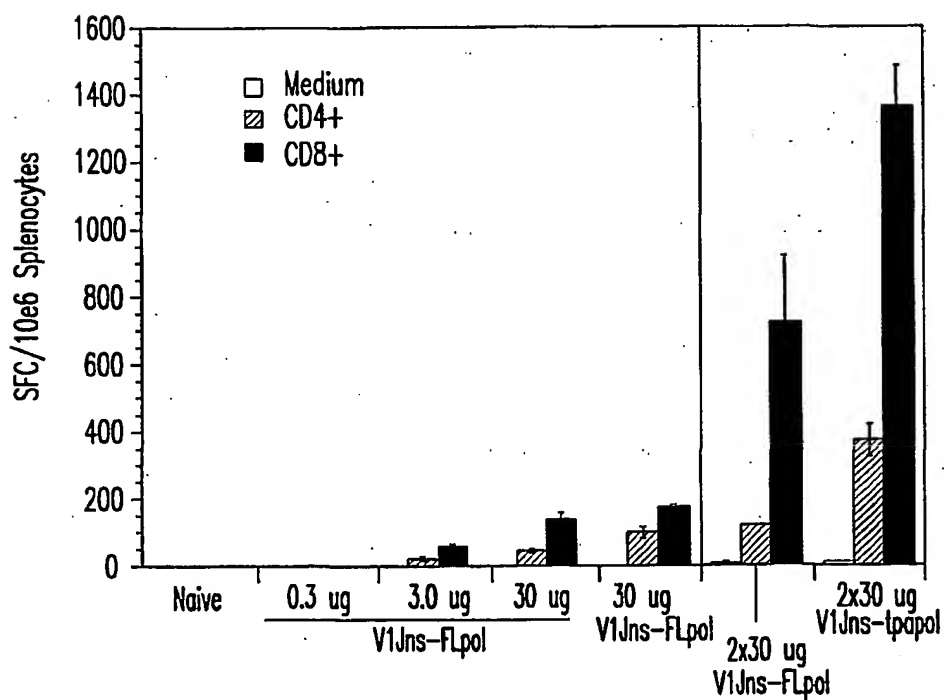


FIG.5

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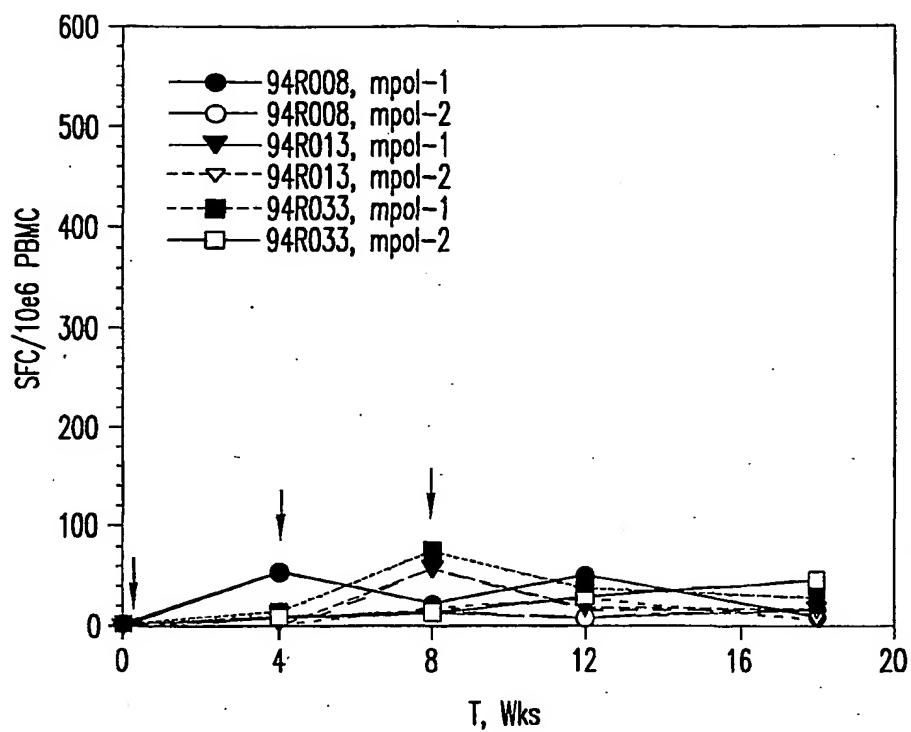


FIG. 6A

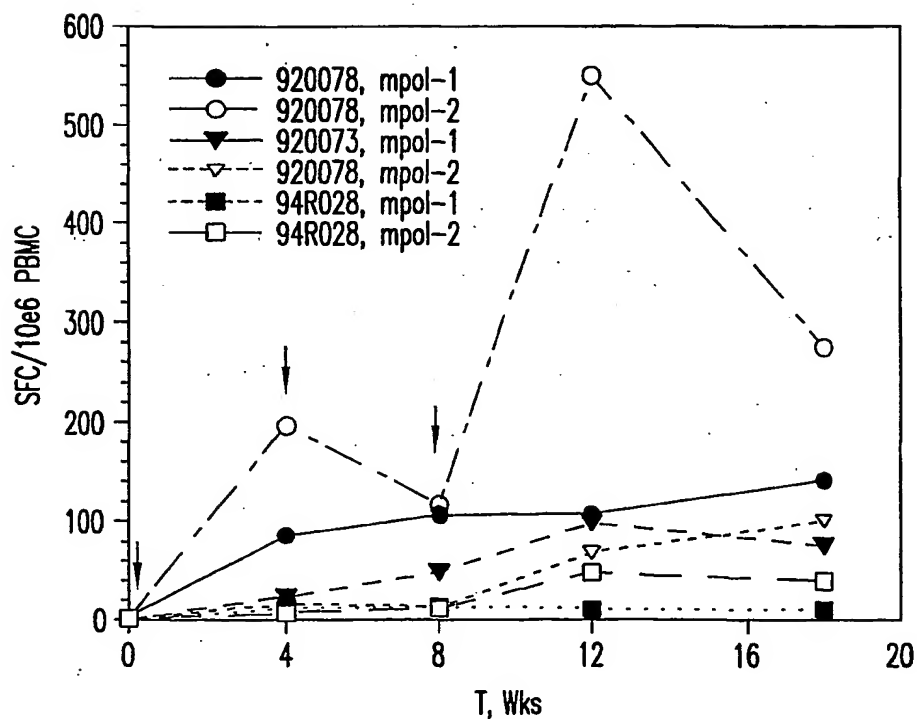


FIG. 6B

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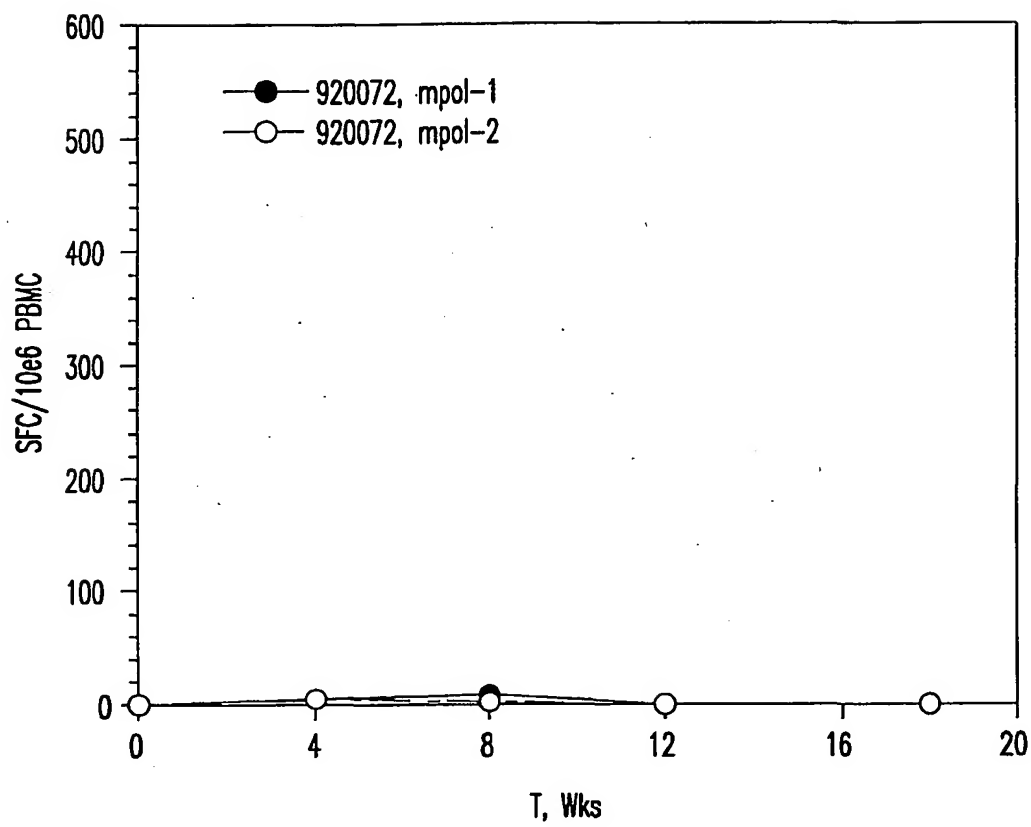


FIG.6C

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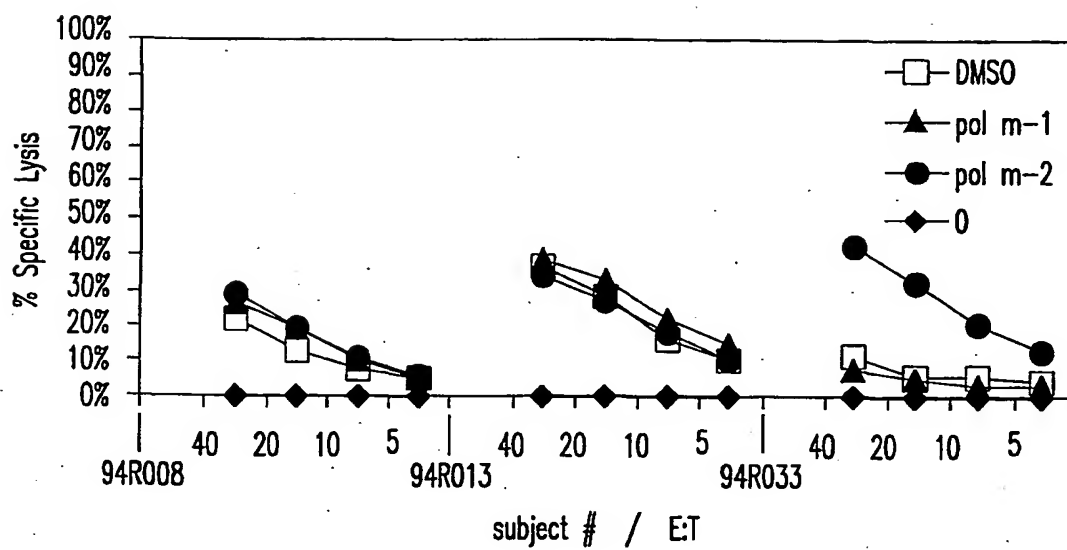


FIG. 7A

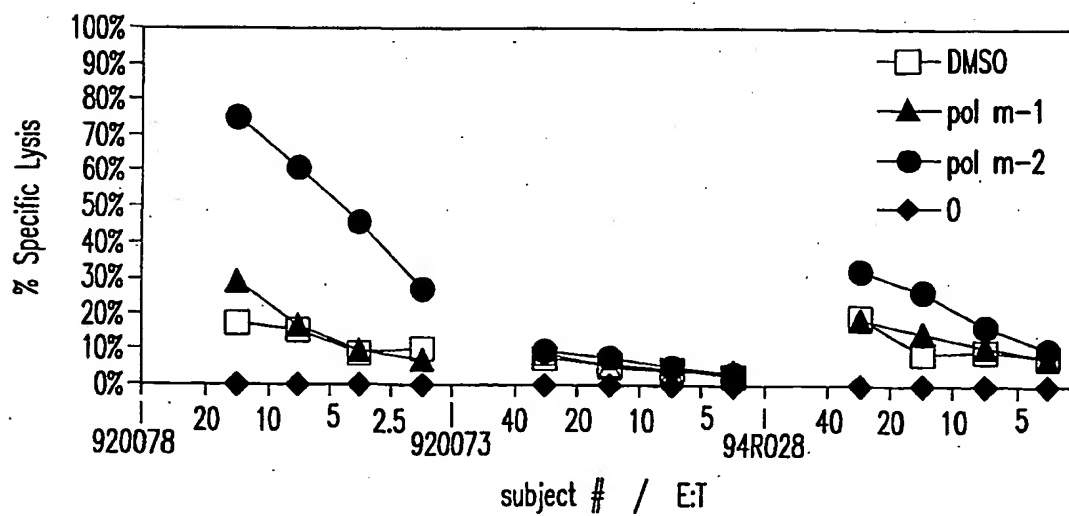


FIG. 7B

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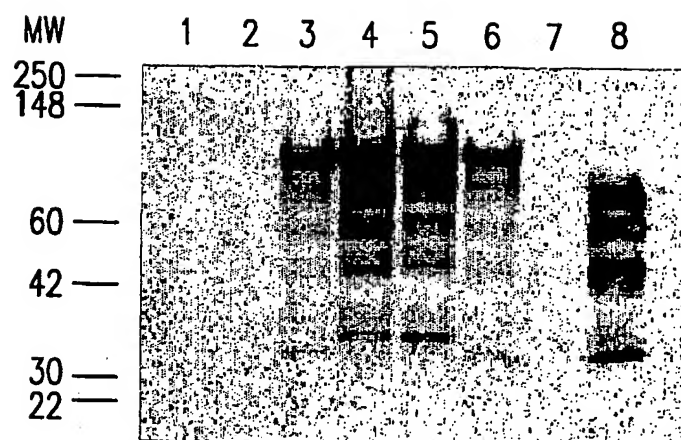


FIG.8

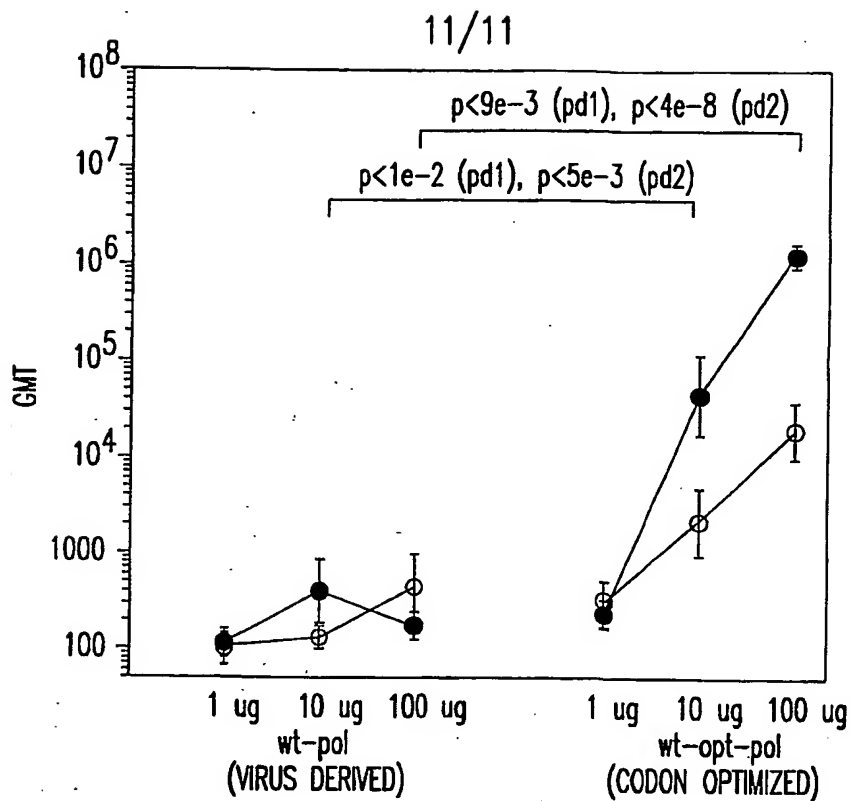


FIG.9

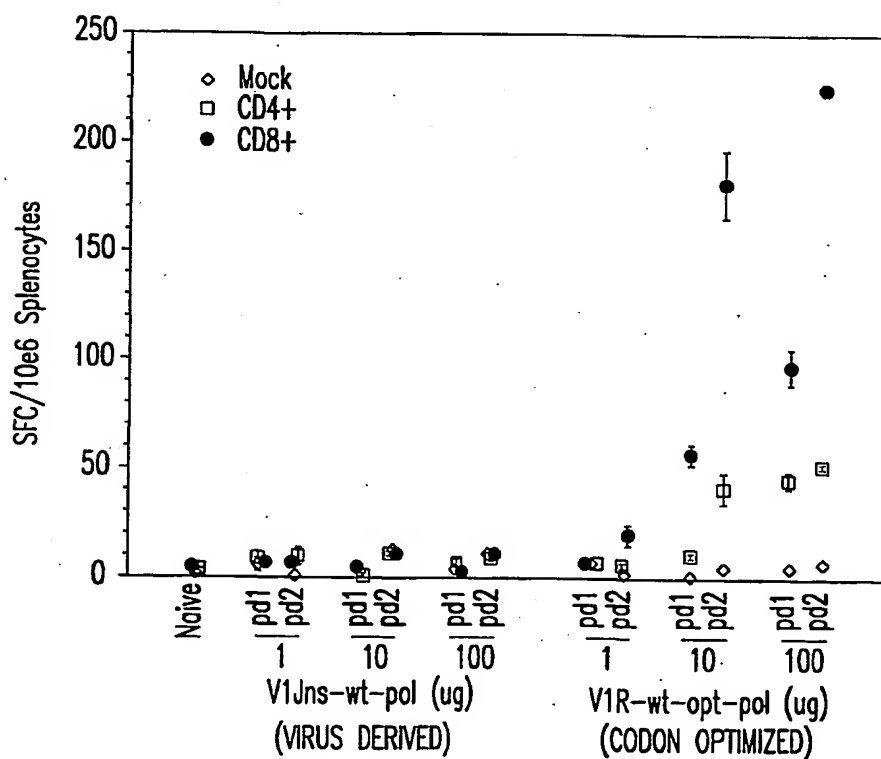


FIG.10

SEQUENCE LISTING

<110> Merck & Co., Inc.

<120> POLYNUCLEOTIDE VACCINES EXPRESSING CODON
OPTIMIZED HIV-1 POL AND MODIFIED HIV-1 POL

<130> 20608Y PCT

<160> 30

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 2577

<212> DNA

<213> Human Immunodeficiency Virus-1

<220>

<221> CDS

<222> (10)...(2562)

<400> 1

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          Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu
            1              5              10

aag cct ggc atg gat ggc ccc aag gtg aag cag tgg ccc ctg act gag      99
Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu
  15              20              25              30

gag aag atc aag gcc ctg gtg gaa atc tgc act gag atg gag aag gag     147
Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu
          35              40              45

ggc aaa atc tcc aag att ggc ccc gag aac ccc tac aac acc cct gtg     195
Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val
          50              55              60

ttt gcc atc aag aag aag gac tcc acc aag tgg agg aag ctg gtg gac     243
Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp
          65              70              75

ttc agg gag ctg aac aag agg acc cag gac ttc tgg gag gtg cag ctg     291
Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu
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Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val
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Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe
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 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp
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 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
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 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly
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 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
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 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
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 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
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Ile	Phe	Gln	Ser	Ser	Met	Thr	Lys	Ile	Leu	Glu	Pro	Phe	Arg	Lys	Gln
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Asn	Pro	Asp	Ile	Val	Ile	Tyr	Gln	Tyr	Met	Ala	Ala	Leu	Tyr	Val	Gly
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Ser	Asp	Leu	Glu	Ile	Gly	Gln	His	Arg	Thr	Lys	Ile	Glu	Glu	Leu	Arg
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Gln	His	Leu	Leu	Arg	Trp	Gly	Leu	Thr	Thr	Pro	Asp	Lys	Lys	His	Gln
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Lys	Glu	Pro	Pro	Phe	Leu	Trp	Met	Gly	Tyr	Glu	Leu	His	Pro	Asp	Lys
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Trp	Thr	Val	Gln	Pro	Ile	Val	Leu	Pro	Glu	Lys	Asp	Ser	Trp	Thr	Val
				245					250					255	
Asn	Asp	Ile	Gln	Lys	Leu	Val	Gly	Lys	Leu	Asn	Trp	Ala	Ser	Gln	Ile
			260					265					270		
Tyr	Pro	Gly	Ile	Lys	Val	Arg	Gln	Leu	Cys	Lys	Leu	Leu	Arg	Gly	Thr
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Lys	Ala	Leu	Thr	Glu	Val	Ile	Pro	Leu	Thr	Glu	Glu	Ala	Glu	Leu	Glu
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Leu	Ala	Glu	Asn	Arg	Glu	Ile	Leu	Lys	Glu	Pro	Val	His	Gly	Val	Tyr
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Tyr	Asp	Pro	Ser	Lys	Asp	Leu	Ile	Ala	Glu	Ile	Gln	Lys	Gln	Gly	Gln
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Gly	Gln	Trp	Thr	Tyr	Gln	Ile	Tyr	Gln	Glu	Pro	Phe	Lys	Asn	Leu	Lys
			340					345					350		
Thr	Gly	Lys	Tyr	Ala	Arg	Met	Arg	Gly	Ala	His	Thr	Asn	Asp	Val	Lys
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Gln	Leu	Thr	Glu	Ala	Val	Gln	Lys	Ile	Thr	Thr	Glu	Ser	Ile	Val	Ile
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Trp	Gly	Lys	Thr	Pro	Lys	Phe	Lys	Leu	Pro	Ile	Gln	Lys	Glu	Thr	Trp
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Glu	Thr	Trp	Trp	Thr	Glu	Tyr	Trp	Gln	Ala	Thr	Trp	Ile	Pro	Glu	Trp
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Glu	Phe	Val	Asn	Thr	Pro	Pro	Leu	Val	Lys	Leu	Trp	Tyr	Gln	Leu	Glu
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Lys	Glu	Pro	Ile	Val	Gly	Ala	Glu	Thr	Phe	Tyr	Val	Ala	Gly	Ala	Ala
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Leu	Gln	Ala	Ile	Tyr	Leu	Ala	Leu	Gln	Asp	Ser	Gly	Leu	Glu	Val	Asn
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Lys	Lys	Glu	Lys	Val	Tyr	Leu	Ala	Trp	Val	Pro	Ala	His	Lys	Gly	Ile
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Gly	Gly	Asn	Glu	Gln	Val	Asp	Lys	Leu	Val	Ser	Ala	Gly	Ile	Arg	Lys
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Val	Leu	Phe	Leu	Asp	Gly	Ile	Asp	Lys	Ala	Gln	Asp	Glu	His	Glu	Lys
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Tyr	His	Ser	Asn	Trp	Arg	Ala	Met	Ala	Ser	Asp	Phe	Asn	Leu	Pro	Pro
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Val	Val	Ala	Lys	Glu	Ile	Val	Ala	Ser	Cys	Asp	Lys	Cys	Gln	Leu	Lys
		595					600					605			
Gly	Glu	Ala	Met	His	Gly	Gln	Val	Asp	Cys	Ser	Pro	Gly	Ile	Trp	Gln
	610					615					620				
Leu	Ala	Cys	Thr	His	Leu	Glu	Gly	Lys	Val	Ile	Leu	Val	Ala	Val	His
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Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
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 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
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 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 675 680 685
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 690 695 700
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
 705 710 715 720
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 725 730 735
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 740 745 750
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 755 760 765
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 770 775 780
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 785 790 795 800
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
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 Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser
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 ccc att gag act gtg cct gtg aag ctg aag cct ggc atg gat ggc ccc 145
 Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro
 35 40 45
 aag gtg aag cag tgg ccc ctg act gag gag aag atc aag gcc ctg gtg 193
 Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val
 50 55 60
 gaa atc tgc act gag atg gag aag gag ggc aaa atc tcc aag att ggc 241
 Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly
 65 70 75
 ccc gag aac ccc tac aac acc cct gtg ttt gcc atc aag aag aag gac 289
 Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp
 80 85 90

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acc cag gac ttc tgg gag gtg cag ctg ggc atc ccc cac ccc gct ggc Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly 115 120 125	385
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ttc tct gtg ccc ctg gat gag gac ttc agg aag tac act gcc ttc acc Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr 145 150 155	481
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ctg tgg atg ggc tat gag ctg cac ccc gac aag tgg act gtg cag ccc Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro 255 260 265 270	817
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caa atc tac cag gag ccc ttc aag aac ctg aag act ggc aag tat gcc Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala 370 375 380	1153
agg atg agg ggg gcc cac acc aat gat gtg aag cag ctg act gag gct Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala 385 390 395	1201
gtg cag aag atc acc act gag tcc att gtg atc tgg ggc aag acc ccc Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro 400 405 410	1249
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cag ggg gtg gtg gag tcc atg aac aag gag ctg aag aag atc att ggg Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly 735 740 745 750	2257
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gct ggg gag agg att gtg gac atc att gcc aca gac atc cag acc aag Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys 785 790 795	2401
gag ctc cag aag cag atc acc aag atc cag aac ttc agg gtg tac tac Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr 800 805 810	2449

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Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val
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gtg ccc agg agg aag gcc aag atc atc agg gac tat ggc aag cag atg      2593
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Lys Gln Trp Pro Leu Thr Glu Lys Ile Lys Ala Leu Val Glu Ile
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Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
65      70      75      80
Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
85      90      95
Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
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Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
115     120     125
Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
130     135     140
Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
145     150     155     160
Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
165     170     175
Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
180     185     190
Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
195     200     205
Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
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His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
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Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
260     265     270
Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
275     280     285
Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
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Leu	Lys	Glu	Pro	Val	His	Gly	Val	Tyr	Tyr	Asp	Pro	Ser	Lys	Asp	Leu
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Ile	Ala	Glu	Ile	Gln	Lys	Gln	Gly	Gln	Gly	Gln	Trp	Thr	Tyr	Gln	Ile
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Lys	Ile	Thr	Thr	Glu	Ser	Ile	Val	Ile	Trp	Gly	Lys	Thr	Pro	Lys	Phe
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Lys	Leu	Pro	Ile	Gln	Lys	Glu	Thr	Trp	Glu	Thr	Trp	Trp	Thr	Glu	Tyr
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Trp	Gln	Ala	Thr	Trp	Ile	Pro	Glu	Trp	Glu	Phe	Val	Asn	Thr	Pro	Pro
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Leu	Val	Lys	Leu	Trp	Tyr	Gln	Leu	Glu	Lys	Glu	Pro	Ile	Val	Gly	Ala
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Glu	Thr	Phe	Tyr	Val	Asp	Gly	Ala	Ala	Asn	Arg	Glu	Thr	Lys	Leu	Gly
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Thr	Asp	Thr	Thr	Asn	Gln	Lys	Thr	Glu	Leu	Gln	Ala	Ile	Tyr	Leu	Ala
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Gly	Ser	Asn	Phe	Thr	Gly	Ala	Thr	Val	Arg	Ala	Ala	Cys	Trp	Trp	Ala
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Gly	Ile	Lys	Gln	Glu	Phe	Gly	Ile	Pro	Tyr	Asn	Pro	Gln	Ser	Gln	Gly
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Val	Val	Glu	Ser	Met	Asn	Lys	Glu	Leu	Lys	Lys	Ile	Ile	Gly	Gln	Val
			740					745					750		
Arg	Asp	Gln	Ala	Glu	His	Leu	Lys	Thr	Ala	Val	Gln	Met	Ala	Val	Phe
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 Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro
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 Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val
 50 55 60
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 Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly
 65 70 75
 ccc gag aac ccc tac aac acc cct gtg ttt gcc atc aag aag aag gac 289
 Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp
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 Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly
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<212> DNA

<213> E. coli (V1Jneo plasmid)

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<211> 4867

<212> DNA

<213> E. coli (V1Jns plasmid)

<400> 16

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/34724

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 48/00; C12Q 1/70.

US CL : 514/44; 435/5; 424/93.1.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 435/5; 424/93.1.

Documentation searched other than *minimum* documentation to the extent that such documents are included in the fields searchedElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Medline, embase, scisearch, biosis, caplus and WEST**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 6,099,848 A (FRANKEL et al) 08 August 2000 (08.08.2000), page 12 paragraph 6.	1-14, 17
Y	WO 97/31115 A2 (MERCK & CO. INC.), 28 August 1997, page 36.	4
X	WO 90/10230 A1 (UNIVERSITY OF OTTAWA) 07 September 1990, page 11.	17
Y	US 5,858,646 A (KANG) 12 January 1999 (12.01.1999), col. 2, lines 10-17	1-14, 17

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

22 February 2001

Date of mailing of the international search report

09 MAR 2001

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

Box PCT

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PARALEGAL SPECIALIST

TECHNOLOGY CENTER 1600

INTERNATIONAL SEARCH REPORT

Internat application No.

PCT/US00/34724

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claim Nos.: 15 & 16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

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